

tropinol with tropic acid that contained an atom of ^{14}C in the position between the benzene ring and the carboxyl group. Mice were the first animals studied (over 100 were used), followed by rats, a few cats, and finally eight human beings. The original group of investigators separated into three parts before the entire series of studies was completed, and one of those parts studied the fates of two differently labeled atropines in man.

The first part of the research to be reported was the study of the metabolic fates of labeled tropic acid and atropine in mice and rats (91,92). Mice and rats given intraperitoneal injections of alpha- ^{14}C tropic acid at 1 mg/kg excreted in their urine only a single labeled compound that had essentially the same chromatographic characteristics as authentic tropic acid. The excretion of ^{14}C administered in this form was complete within about 2.5 h in the mice and within slightly more than 3 h in the rats.

When the labeled atropine was administered to both mice and rats by both intravenous and intraperitoneal injections, the cumulative excretion of ^{14}C in the urine by the mouse was always considerably greater than that by the rat. The label from alpha- ^{14}C atropine injected intravenously into mice appeared in their urine slightly more promptly and to a somewhat greater extent than that from labeled atropine that had been injected intraperitoneally or subcutaneously or that had been administered by gavage. The curve for the clearance of ^{14}C from the body of the mouse with time elapsed after subcutaneous injection of the labeled alkaloid required three simultaneous exponential equations to represent the observations after a period of latency of about 45 min, during which excretion of ^{14}C followed none of the three exponential relationships. In addition to atropine itself, at least three other substances containing ^{14}C appeared in the urine of the mouse.

At 30 min after intravenous injection of labeled atropine into mice, the structures found to have the highest concentrations of ^{14}C were the gall bladder and the bile, the urinary bladder, and the duodenum and jejunum. By 4 h after injection, the structures with the highest concentrations of ^{14}C were the gall bladder and the bile, the urinary bladder, and the ileum. At 24 h after injection, the structures with the highest concentrations of ^{14}C were the colon and cecum, the gall bladder and the bile, and the stomach. (In all cases, the hollow viscus and its contents are designated by the name of the viscus alone.) The comparatively high concentration of the label in the stomach 24 h after injection may indicate that the mice had ingested some of their feces, the contents of the colon and cecum having high concentrations of ^{14}C at that time. No $^{14}\text{CO}_2$ was found in the expired air.

It is clear that the principal routes of removal from the body of the atropine label used by Gosselin et

al. (91, 92) were the urine and the feces, the intestinal route of excretion being comparatively minor. Whatever the mode of administration of the labeled atropine to mice, 80-90% of the ^{14}C was excreted in the urine. The consistent presence of the gall bladder and its contents among the structures with the highest concentrations of the label indicates that the label enters the gastrointestinal tract after secretion by the liver into the bile, which then flows into the small intestine through the bile duct and the common duct. The progressive shift of the segment of the intestinal tract with the highest concentration of ^{14}C from the duodenum and jejunum to the ileum and then to the large bowel reflects the movement of a bolus of an especially high concentration of the isotope down the tract during a period of about 24 h.

The failure to find $^{14}\text{CO}_2$ in the expired air of the mice indicates that the tropic acid moiety in the atropine molecule is not metabolized—in accord with the finding that labeled tropic acid itself was excreted without loss in the urine. The finding of atropine but no tropic acid in the urine of the mice indicates that hydrolysis of the ester did not occur rapidly. The latter finding was somewhat unexpected because an esterase capable of hydrolyzing atropine is present in the livers of many vertebrate species, although it or a similar enzyme appears in the sera of only a few species (93-95). This enzyme is also able to hydrolyze L-hyoscyamine, tropinyl benzoate, and caramiphen (95).

The rat was found (96) to excrete in its urine substances containing ^{14}C derived from the labeled atropine with approximately the same paper chromatographic retardation factors (R_f s) as those found previously in the urine of the mouse. The rat differed from the mouse in excreting about 40% of the label in its feces, compared with about 10% for the mouse. The guinea pig, unlike the other mammals studied, excreted in its urine a large part of the ^{14}C in a form that was probably tropic acid or some similar molecule (R_f slightly below that for tropic acid added to urine from the guinea pig). The majority of the ^{14}C in the urine of the cat was excreted in the form of atropine, only two other small peaks of radioactivity being evident on paper chromatograms. The rat and the cat, unlike the mouse and the guinea pig, excreted almost as much of the label in feces as in urine. In the rat and the cat, the livers were more active both in initial uptake and in maintaining a high uptake until 4 h after intravenous injection of labeled atropine than those of the mouse and the guinea pig. Increased transfer of the isotope from the blood into the bile by the liver in the former two species probably explains its greater fecal excretion by these animals than by the other two species.

Despite the quantitative differences in excretion of labeled atropine by different routes in the mouse and the rat, Gabourel and Gosselin (97) proposed that atropine

follows the same metabolic pathways in the rat as in the mouse. They studied mice given intravenous injections of alpha-[^{14}C]atropine and found that approximately 25% of the dose was excreted in the urine as atropine, more than 50% as glucuronides, and the remainder as intermediate oxidation products and, probably, tropic acid esters with altered tropanyl moieties. Although they were unable to prove that the aromatic ring of the tropic acid moiety was hydroxylated, they proposed that hydroxylation of this ring, initially at the 4-position and then at the 3-position, resulted eventually in the production of monoglucuronides and diglucuronides. However, Phillipson *et al.* (98) found that incubation of atropine or hyoscyne with a preparation of microsomes from guinea pig liver led first to the dealkylation of the nitrogen atom of the tropanyl or the scopyl moiety and sequentially to production of the *N*-oxides and *N*-hydroxynoratropine or *N*-hydroxynorhyoscyne. It seems possible that conjugation with glucuronic acid from uridine-5-diphospho-alpha-D-glucuronic acid under the catalytic influence of uridine diphosphate glucuronyl transferase occurred in the course of the oxidative series of reactions mentioned earlier and resulted in the formation of an *N*-glucuronide of noratropine. Such a reaction is known to occur with secondary aromatic amines, such as noratropine.

Werner and Schmidt (99) studied the types of chemical reactions undergone by [^{14}C]atropine introduced into mice, rats, guinea pigs, rabbits, cats, and monkeys. Their general conclusion was that, although various species differed quantitatively in the relative amounts of particular end products of atropine metabolism, the chemical changes undergone by the molecule in the various species were the same: hydrolysis of the ester linkage, hydroxylation of the benzene nucleus, glucuronidation, and oxidation to CO_2 .

Kalser *et al.* (100) found that bile of rats into which [^{14}C]atropine had been injected intravenously might contain up to five peaks of radioactivity of comparatively low *R_f*: 0.02, 0.08-0.09, 0.16, 0.22-0.23, and 0.68. The substances represented by *R_f*s of 0.08-0.09 and 0.16 were present in the bile in the highest concentrations. No tropic acid or unaltered atropine was found in bile. Metabolites of atropine appeared in the bile within 10 min after intravenous injection of that alkaloid. The plasma of these rats contained only unchanged atropine in concentrations great enough to be detectable.

Isolated, perfused livers of rats exposed to [^{14}C]atropine injected into the perfusion fluid excreted about 60% of the ^{14}C into the bile during 4 h (101). All the metabolites were more polar than atropine itself, but they were not identified.

Winbladh (102) presented data on the distribution of atropine labeled randomly with ^3H in newborn pups,

pups at 3 and 6 wk of age, pups at 3 mo of age, and adult dogs (10 mo old or older). After subcutaneous injection of labeled atropine at 0.5 mg/kg, the isotope was removed from the blood plasma much more slowly in the newborn pups than in the adult animals. The pups of other ages had intermediate half-times for removal from the plasma, the greatest step of difference being between newborn pups and those 3 wk old. The half-times for removal of the label from the plasma after intravenous injection of labeled atropine were not strikingly different from those measured after subcutaneous injections.

In newborn pups, the organs that initially took up the highest concentrations of the label were the kidneys and the liver, the kidneys having a higher concentration. The liver maintained a higher proportion of its early concentration of ^3H until 8 h after the injection than the kidneys, however. Pups 6 wk old behaved qualitatively like the newborn ones, but in the adult dogs the initial uptake of the label by the liver was considerably greater (by up to 80%) than that by the kidneys. Of the ^3H in the urine, 50-90% was in the form of unchanged atropine. In brain, Winblad (102) found a progressively decreasing concentration of atropine (the only form in which the label was found in brain) as serial samples were taken from the cortex to the vicinity of the lateral ventricles.

Harrison et al. (103) used atropine labeled with ^3H in the para position of the benzene ring in the tropic acid moiety of atropine to follow the disposition of that alkaloid in the rat. They found that the mean half-times for removal of the label from various tissues after intraperitoneal injection were as follows: plasma, 44 min; heart and kidney, 54 min; liver, 74 min; brain, 76 min; and fat, 101 min. The half-times were determined from the slopes of the decay curves.

Two fairly recent papers on the metabolic fate of atropine in man have been published (104,105); an excellent summary of the work was presented by Kalser (106). The two subjects in the first of these papers excreted within 24 h 85% of an intramuscularly injected dose of 2 mg of alpha- ^{14}C atropine. A urine sample collected from one of the subjects between 1.5 and 4 h after injection contained substances that produced four small, interconnected peaks of radioactivity on a paper chromatogram that were apparently removed by incubation of the urine with bacterial beta-glucuronidase. Only three clear peaks of radioactivity appeared on the paper chromatograms of this urine after it had been exposed to glucuronidase. Two of these had R_f s that agreed with those for atropine (the major peak) and for tropic acid (a small peak). The identity of the third peak, with an R_f below that for atropine, was not determined. After alkaline hydrolysis of this sample of urine, the only radioactive substance detected in the chromatogram had an R_f similar to that of tropic acid; thus, the tropic acid

portion of the ester seems not to have been altered metabolically to any important extent.

The second paper (105) used two samples of [^{14}C]atropine; one was labeled on the methyl group attached to the nitrogen atom of the tropine moiety, and the other was labeled at the two carbon atoms in the ring structure of the tropine moiety adjacent to the carbon atom involved in the ester linkage. Two male and two female subjects received intramuscular injections of 2 mg of one of these labeled atropines. One subject received individual injections of each labeled atropine on two different days. When the methyl-labeled atropine was administered, $^{14}\text{CO}_2$ appeared in the expired air. Administration of the other labeled atropine did not result in the excretion of $^{14}\text{CO}_2$, nor had this substance been found in the air expired by the only one of the subjects in the first of these papers (104) whose expired air was examined. One concludes, therefore, that the only part of the atropine molecule that is subject to complete oxidation is the methyl group on the nitrogen atom of the tropine moiety. As for excretion of the label in the urine, the two samples of atropine used by Kalser and McLain yielded similar results. There was rapid excretion of ^{14}C during the first 4 h after injection that was nearly linear with time, but by 16 h after injection the rate of excretion of ^{14}C in the urine had decreased to a much lower rate that was also nearly linear with time. The low rate persisted for up to 48 h after injection in the one subject who received each of the labeled atropines on separate days.

Kalser and McLain (105) found that glucuronidation was an important metabolic pathway in the early excretion of atropine from the body, but that this type of reaction seemed not to be particularly important after 4 h from the time of injection of atropine. Referring to the work of Phillipson *et al.* (98), one may suppose that glucuronidation occurs when noratropine is produced by oxidative dealkylation of the nitrogen atom of the tropine moiety of atropine. The paper by Kalser and McLain indicated that excretion of $^{14}\text{CO}_2$ in the expired air reached a peak about 75 min after the intramuscular injection of atropine and suggested that by 4 h after such a dose it may have been fairly low again. Possibly, therefore, glucuronidation and oxidative dealkylation proceed contemporaneously and in a coupled fashion.

The two labeled atropines used by Kalser and McLain (105) yielded the same chromatographically distinguishable ^{14}C -containing substances in the urine excreted by their subjects. There were four principal peaks of radioactivity, all of which appeared to consist, at least in part, of glucuronides at the early times of collection of urine. Peaks of radioactivity with R_f s similar to those of labeled atropine and tropine were the most prominent ones in the paper chromatograms of the

urine of these subjects. In samples of urine collected during the first 6 h after a dose of labeled atropine, a component of Rf 0.70 seemed to be converted to one of Rf 0.55 by incubation with beta-glucuronidase from beef liver.

In summarizing this work on the metabolism of atropine by man, Kalser (106) used Tonnesen's data (89) on the urinary excretion of atropine by five subjects to show that there is a fairly broad range of rates of excretion of atropine; during the 12 h after ingestion of atropine, these five subjects excreted 15.5-42% of the ingested dose in their urine. It is not surprising, therefore, that one subject given labeled atropine intramuscularly excreted only 35.3% of the injected dose during the first 8 h, whereas the five experiments reported by Kalser and McLain (105) yielded cumulative excretion of injected atropine in urine during the same period between about 63% and 112% of the injected amount. After omission of the highest value, which came from urine samples that the investigators thought might have been handled improperly, there is still a variation between 63% and 83%. (Three experiments with three different subjects yielded closely similar excretion rates, with a mean of about 80% of the injected dose. A fourth experiment with one of the same subjects yielded the low value of 63%.)

When the data on ^{14}C still in the body at various times after intramuscular injection of tropine-labeled atropines were plotted and analyzed to yield two linear regressions in time, the derived half-times ranged between 1.3 and 2.1 h for the early parts of the curves and between 12.5 and 38.0 h for the later parts. When the data for the subject studied most extensively by Gosselin et al. (104) after intramuscular injection of tropine-labeled atropine were treated similarly, the half-times were 1.8 and 16.5 h. Apparently, the two types of labeling led to very similar estimates of the rate of removal of atropine from the body.

Two other studies provide somewhat similar findings. In one (107), coma-producing doses of atropine sulfate were injected intravenously, [^3H]atropine being mixed with unlabeled atropine to permit a dose of 100 μCi of ^3H to be included in the total dose of atropine sulfate. One psychopath, one epileptic, and 13 schizophrenics were studied. Graphs of data from three patients were presented in the paper. One man received a total dose of 70 mg of atropine sulfate (1.06 mg/kg), another man received 150 mg (2.8 mg/kg), and the third patient, a woman, received 270 mg (4.5 mg/kg). All injections were said to have been intravenous, but the curve for the concentration of the isotope in the blood of the woman is like that from a subcutaneous injection. During the 24 h after injection, the recoveries of the label in the urine, from the lowest dose to the highest, were 27.1%, 48.0%, and 81.1%. The concentrations of the isotope in the blood of the two men decreased rapidly during the first 15 min and more slowly thereafter; in the

woman, the concentration increased to a maximum at about 15 min after the injection and then decreased moderately slowly. In all three patients, the concentration of the isotope in the blood became zero by or soon after 24 h after injection.

Hayden *et al.* (108) used a radioimmunoassay method to measure the removal of atropine from human plasma in four subjects given intravenous injections of 0.32 mg of atropine. They found a very rapid decrease in the plasma concentration during the first 5 min after injection, followed by a slower decrease. By 4 h after injection, the atropine concentration in the plasma was only about 2.25% of that present 5 min after injection. The initial period of rapidly changing concentration of atropine in the plasma may be a period of mixing in the plasma and equilibration between the plasma and various tissues.

A paper by Virtanen *et al.* (109) presented graphs of concentration of atropine in the serum of two women given 1.3 mg of atropine intravenously. There was an initial very rapid decrease in concentration, estimated by a radioimmunoassay method, followed by a slower decrease. The half-times for removal of atropine from the blood in these two women were 2.09 and 1.86 h. These times are similar to those calculated by Kalser and McLain (105) for the early parts of their curves. The half-times in the paper by Gaszner *et al.* (107) correspond more nearly with those calculated by Kalser and McLain for the later parts of their curves.

Because tropic acid was the only substance found after alkaline hydrolysis of urine from subjects given tropate-labeled atropine and a substance that was either tropine or a close relative was the only one found in large quantities after alkaline hydrolysis of urine from subjects given tropine-labeled atropine, it is apparent that atropine did not undergo drastic metabolic modification in human subjects. Oxidative dealkylation, with excretion of $^{14}\text{CO}_2$ in the expired air, probably led to the temporary formation of noratropine. This compound may then have been glucuronidated directly or been oxidized further to the hydroxylamine form and then glucuronidated. The finding that glucuronidation became a relatively unimportant reaction within about 4-6 h after the injection of atropine into muscle suggests that there is some sort of coupling between oxidative dealkylation of the tropine moiety of atropine and glucuronidation; in other words, glucuronidation may depend on the presence of some particular concentration of substrate, whether that substrate be noratropine or hydroxynoratropine.

HUMAN DATA FROM THE GENERAL LITERATURE

Gordon and Frye (27) collected from the literature reports on 11 deaths of humans considered to be due to atropine. Five of these involved application of solutions of atropine to the eyes--one person was thought to have received 1.6 mg of atropine and another 18.1 mg.

Four deaths were related to ingestion of atropine. One person had taken 198 mg of atropine with 100 mg of morphine. A 3.5-yr-old child had taken 540 mg of atropine and 1,350 mg of phenobarbital. Another person was reported to have taken a daily dose of tincture of belladonna equal to 1.2-2.5 mg of atropine for 6 yr before death (probably the highest dose for most of the time); there was nothing in the report to indicate clearly that death was attributable to long-term ingestion of atropine (110). Two deaths were connected with injection of atropine, in one case of 32 mg. On the other side of the picture, Alexander *et al.* (111) reported that an adult had survived after ingesting 1,000 mg of atropine; Mackenzie and Piggott (112) told of three children weighing 14-24 kg who lived after taking 600 mg of atropine.

Reports of cases of serious, nonfatal intoxication by tropic acid esters indicate that recovery from such episodes is reasonably rapid. For example, Sims (113) reported the case of a 67-yr-old woman who had applied belladonna plasters to her back for a month to relieve backache. During this time, the skin to which the plasters had been applied became excoriated. On the day of admission to a hospital, the patient had disturbed her husband in the early morning by wild and incoherent mutterings. On admission, she had two plasters on her back and was delirious and flushed. Her pupils were dilated equally and unreactive to light. Her arms were held in flexion and the legs in extension. After removal of the plasters from her back, she regained consciousness within about 12 h. Her pupils remained dilated for more than 36 h, however. She was released from the hospital as fully recovered, including healing of her skin, on the tenth day after admission.

Intoxication by scopolamine with similar signs was reported by Beach *et al.* (114). A 35-yr-old woman had taken 12 Sominex tablets (equivalent to 3 mg of scopolamine). She was in stupor with occasional clonic convulsions. Her pupils were dilated, her mouth was dry, her skin was hot to touch, her pulse was rapid, and she reacted vigorously to minor stimuli. When she was put to bed, her hands and fingers made repetitive plucking movements at the bedsheet. Apparent visual hallucinations, evidenced by avoidance movements, accompanied by screaming and severe disorientation began to abate after 12 h. The patient was discharged on the fourth day after admission to a hospital.

Stoll (26) compared the actions of atropine and methylatropinium nitrate on several functions of the human body. Methylatropinium nitrate at 10 µg/kg produced mean increases (four subjects) in pulse rate twice those produced by the same dose of atropine. In a series of experiments in which subjects were given identical doses (20 µg/kg) of the two drugs, they were found to have almost identical effects on pulse rate. Methylatropinium nitrate induced somewhat greater increases in the mean

systolic and mean diastolic blood pressures and a considerably greater increase in the minute volume of the heart than atropine. Atropine increased the consumption of oxygen, whereas methylatropinium nitrate decreased it. Methylatropinium nitrate was about twice as effective as atropine in increasing skin temperature.

Cullumbine *et al.* (115) studied the effects of atropine sulfate on healthy men. Twenty men each received 1 mg of atropine sulfate by subcutaneous and intramuscular injection and 0.5 mg by intravenous injection. Forty men received double doses by the same three routes of administration. The first sign of action by atropine in many subjects was a slight, temporary decrease in heart rate, followed by a gradual increase. Intravenous injection produced effects more rapidly than the other routes of administration. The increase in heart rate induced by 1 mg of atropine sulfate injected intravenously was similar to, but of shorter duration than, that induced by subcutaneous or intramuscular injection of 2 mg. Subcutaneous injection of atropine sulfate induced acceleration of the pulse more rapidly, but less lastingly, than the same dose injected intramuscularly.

All of 44 men given 5 mg of atropine sulfate complained of dryness of the mouth and throat, of dizziness, giddiness, or light-headedness, and of difficulty in reading. Other complaints, in order of diminishing frequency, were of difficult urination; of tiredness, lassitude, or sleepiness; of headache or eyache; and of nausea. After 3 mg of this drug, the only complaint voiced by 12 men so treated was of dryness of the mouth and throat; after 2 mg of atropine sulfate, only 37 of 45 subjects complained of dryness of the mouth and throat. The most definite objective effect of atropine was an increase in heart rate. Systolic blood pressure tended to decrease slightly and diastolic pressure to increase. There were also slight, but insignificant, changes in the concentrations of hemoglobin, leukocytes, and blood glucose. Some subjects had changes in their electrocardiograms that indicated sinus arrhythmia, P-wave enlargement, premature atrial contractions, and flattened, coved, or inverted T waves. Daily injections of 2 mg of atropine repeated for 5 d decreased either the severity of or the subjective response to the changes in secretion by the glands of the mouth and in accommodation of the eyes to light and to nearness.

Wyant and Dobkin (116) compared atropine, L-hyoscyamine, and scopolamine for ability to stop salivation induced by intravenous injection of a mixture of carbachol and epinephrine. Scopolamine was slightly more active in this regard than L-hyoscyamine, and both were considerably more active than atropine.

Bachrach (117) surveyed the literature related to a group of anticholinergic drugs, including atropine and methylscopolammonium bromide, and reported the results of studies of small groups of patients. Atropine was about 4

times as active a sialolytic drug as methylscopolammonium bromide, but produced more side effects. In a group of 11 subjects given 0.6 mg of atropine orally, nine complained of side effects; blurred vision was the most common of these; one subject complained of dysuria, and another of nervousness, dizziness, and anorexia. Only one of eight patients given about 2.4 mg of methylscopolammonium bromide complained of blurred vision, and one complained of dysphagia.

Herxheimer (118) compared a small group of anticholinergic drugs--including atropine sulfate, scopolamine hydrobromide, and methylscopolammonium bromide--for ability to affect several functional systems of man after subcutaneous injection. Scopolamine hydrobromide (0.2-0.8 mg) was strikingly more active than either methylscopolammonium bromide (0.125-1.0 mg) or atropine (0.5-2.0 mg) in inducing dilatation of the pupil and paralysis of accommodation. Methylscopolammonium bromide was more potent than scopolamine hydrobromide, which was more potent than atropine, in inhibiting secretion of saliva. The two salts of scopolamine were equally potent, and more so than atropine, in slowing urination. Methylscopolammonium bromide was strikingly more potent than atropine sulfate in increasing heart rate. The incidence of drowsiness was 100% with scopolamine hydrobromide, about 25% with methylscopolammonium bromide, and lowest with atropine. The effects of methylscopolammonium bromide and atropine sulfate on heart rate became evident (18-19 min) and reached their greatest intensities (37-40 min) at about the same times, despite the fact that the dose of methylscopolammonium bromide was only 22.5% that of atropine sulfate. When equally effective doses of the compounds were administered, methylscopolammonium bromide was slower than scopolamine hydrobromide and atropine sulfate in exerting its maximal effect on salivation and on the iris. The effects of scopolamine hydrobromide on the iris and on accommodation for near vision lasted considerably longer than those of equal doses of methylscopolammonium bromide and atropine sulfate.

Using salivary secretion as the indicator, Mirakhur (118) found that atropine and scopolamine were about 2 and 4-5 times as active, respectively, after intramuscular injection as after ingestion. When heart rate was used as the indicator, both alkaloids were about twice as active after intramuscular injection as after ingestion. When the secretion of sweat was used as the indicator, however, ingested scopolamine was nearly as active as that injected intramuscularly, whereas atropine was about twice as active when ingested as when injected.

In a combination of literature review and reports of personal experiences with patients, Schweitzer and Mark (120) summarized the effects of atropine on the intracardiac conduction system and the myocardium. The chronotropic effects of atropine are the best-known ones

on the heart, consisting of an early and brief (a few minutes) slowing of the heart, most apparent with small doses or with doses administered by routes likely to yield slow entrance of the substance into the body, and a later and predominant increase in heart rate. The predominant action is due largely to blockade of vagal impulses to the heart, but is contributed to by improved sinoatrial conduction, increased frequency of discharges by the atrioventricular node, and facilitation of conduction through that node. Atropine may affect the refractory period of atrial muscle and conduction by the His-Tawara bundle, but these actions are uncertain. Atropine may induce atrial fibrillation, atrial ventricular dissociation, ventricular tachycardia, and even ventricular fibrillation. The last result is particularly likely (122,123) when the work of the heart is increased suddenly under conditions of hypoxemia, as when atropine is administered intravenously to an apneic person who has just been overcome by exposure to an inhibitor of cholinesterases and whose heart still beats.

Despite the reputation of scopolamine for causing amnesia, Hardy and Wakely (124) found that only 13 of 100 patients given subcutaneous injections of hyoscine at about 6.3 $\mu\text{g/kg}$ and morphine at about 157.2 $\mu\text{g/kg}$ had any amnesia of being shown a card just before induction of anesthesia. Only seven of these patients had complete amnesia of preoperative events. When atropine at about 9.4 $\mu\text{g/kg}$ was substituted for scopolamine in the preanesthetic mixture, only one of 100 patients had partial amnesia of being shown a card before induction of anesthesia.

Derwent and Karacan (125) examined the effects of intramuscular injections of atropine sulfate (15-60 $\mu\text{g/kg}$), scopolamine hydrobromide (1.5-6 $\mu\text{g/kg}$), and methylscopolammonium bromide (1.5-6 $\mu\text{g/kg}$) on rapid-eye-movement (REM) sleep and its neurogenic correlate, nocturnal penile tumescence (NPT). The drugs were injected 2 h after onset of sleep. Fifteen healthy male volunteers between the ages of 21 and 35 were used. Scopolamine at 6 $\mu\text{g/kg}$ and atropine at 60 $\mu\text{g/kg}$ suppressed both REM sleep and NPT. Quaternized scopolamine had neither of these effects.

Ostfeld *et al.* (126) gave oral doses of 10 mg of atropine sulfate in 100 ml of water to 10 subjects and recorded the rate of salivation, the grades in the seven moods of the Clyde Mood Scale, the electroencephalogram, and EEG arousal induced by single and repetitive flashes of light before and after drug administration. Atropine increased heart rate by 60% and decreased the amount of saliva produced during 1 min by 68%. Five subjects complained of mild difficulty in urinating. These effects were maximal at 2 h after atropine was ingested, as were those on EEG arousal by flashes of light. The last effects consisted of decreasing the duration of the arousal induced by single flashes (by 60.6%) and repeated

flashes (by 57.1%). There was a slight tendency for arousal by repeated flashes of light to be affected before that by single flashes. In the spontaneous EEG recordings, waves of lower amplitude and longer duration (5-8 per second) replaced the normal waves at 8-12 per second, beginning at about 30-60 min after atropine ingestion. Observation of spontaneous behavior and evaluation of mental status and mood revealed that atropine shifted behavior in the direction of reduced energy, decreased voluntary speech and motion, reduced attention span, impaired memory of contemporaneous events, and drowsiness. The changes in the spontaneous EEG and in behavior appeared at about the same time.

Ostfeld and Aruguete (127) performed a similar study in which 54 volunteers were subjected to subcutaneous injections of 150-800 ug of scopolamine hydrobromide. The lowest dose induced moderate bradycardia, but decreased salivation. The highest dose completely blocked the secretion of saliva and seemed to induce sleep, hallucination, and mental disorientation more frequently than the dose of 10 mg of atropine sulfate used by Ostfeld *et al.* (126). The larger doses were associated with decreased ability to perform tasks requiring close attention.

Commin *et al.* (128) reported that intravenous injection of physostigmine sulfate at 40 µg/kg into six patients in coma as a result of administration of atropine was capable of decreasing the depth of the coma, but that the duration and extent of the change were variable. The treatment could be repeated, however. In a later paper, the same group of investigators (129) reported that treatment with physostigmine in six cases of attempted suicide with anticholinergic preparations could be monitored with EEG recordings, using EEG alerting as a signal of effect. When the preparations used included overdoses of drugs other than atropine or other anticholinergic substances, physostigmine had no effect. They pointed out that therapy for anticholinergic coma with physostigmine was limited by the short period of its activity and by its secondary effects, including premature ventricular contractions and convulsant activity.

Hollender *et al.* (130) reported on a woman who complained of auditory and visual hallucinations after taking two Somnex tablets. It developed that she had also taken chlorpromazine, which has distinct anticholinergic activity, and amitriptyline, which has the greatest anticholinergic activity of any of the common tricyclic antidepressants. The woman's pupils were markedly dilated and fixed and did not constrict when drops of a 1% solution of pilocarpine were applied to the eyes. Eventually, she was found to have been using eyedrops of tropicamide, a potent but nonpersistent mydriatic. Shortly after admission to the hospital, the woman had a seizure. This was thought to have been of psychogenic origin; there were no recurrences. With no

drug treatment and prevention of any access to drugs by the patient, her disorientation cleared rapidly. (She claimed, however, that hallucinations persisted during her stay in the hospital of 2 wk, but the investigators considered that she was probably attempting to prolong her hospitalization.)

Aucamp and Meyer (131) reported that well-controlled epileptics may experience sudden exacerbation of their seizures if they begin taking drugs with anticholinergic activity (as in some antacid preparations) or antihistaminic activity (as in preparations for amelioration of colds and influenza).

Moskovitz et al. (132) analyzed the hallucinations experienced by a group of 88 patients, 32-84 yr old, with idiopathic parkinsonism for at least 6 mo. The therapeutic regimens when these patients began to hallucinate had included anticholinergic drugs with or without amantadine (13.9%), alone (23.3%), and with amantadine or anticholinergic drugs or both (62.8%). The hallucinations had been visual for 87.1% of the patients, auditory for 35.5%, and tactile for 4.8%; 25.8% had both visual and auditory hallucinations. The hallucinations were nonthreatening for 72.7% of the patients and threatening for 27.3%. The investigators believed that anticholinergic drugs probably were the principal factors in giving rise to hallucination.

Holland et al. (133,134) gave men intramuscular injections of sterile water, 2 mg of atropine sulfate with 500 mg of N-methyl-2-hydroxyiminomethylpyridinium methyl sulfonate (P2S), 750 mg of P2S, 750 mg of P2S with 2 mg of atropine sulfate, or 2 mg of atropine sulfate. The initial rate of absorption was judged by the time after injection required for attainment of the maximal reduction in heart rate. Five volunteers were used to examine the effects of water, 750 mg of P2S, 750 mg of P2S with atropine sulfate, and atropine sulfate alone; seven were used to determine the effects of 750 mg of P2S with atropine sulfate and atropine sulfate alone; 10 were used to compare the effects of 500 mg of P2S with atropine sulfate and atropine sulfate alone.

EEGs were recorded after the subjects had reclined quietly on beds for an hour. The injection was then given into the upper outer aspect of the thigh, and ECG recording continued for about 2 hr. The air temperature was 21-24°C. All 22 volunteers had control ECGs recorded on day 1. After 2 or 3 d, volunteers 1 to 12 were given 2 mg of atropine sulfate; 5 d later, they received a mixture of 2 mg of atropine sulfate and 750 mg of P2S. Five of these volunteers were given sterile water 2 d after the injection of the mixture of atropine and oxime; a day later, these five received 750 mg of P2S. The 10 remaining volunteers received injections of 2 mg of atropine sulfate and 5 d later of 2 mg of atropine sulfate and 500 mg of P2S.

The heart rates of the volunteers given no injection, injections of sterile water, or injections of 750 mg of P2S had closely similar variations with time. The graphs of heart rate after injections of 2 mg of atropine sulfate, of 2 mg of atropine sulfate and 500 mg of P2S, and of 2 mg of atropine sulfate and 750 mg of P2S followed closely similar courses with time after injection. These were distinctly different from the first set of lines. One can conclude that 750 mg of P2S alone had no significant effect on heart rate and that addition of either 500 or 750 mg of P2S to 2 mg of atropine sulfate had no striking effect on absorption of atropine from a site of intramuscular injection. The initial rate of absorption of atropine seems to have been increased slightly by both doses of P2S (mean time to maximal bradycardia was decreased from 18.7 min to 12.9 min by 500 mg of P2S and from 15.3 min to 12.2 min by 750 mg of P2S).

Martin (135) followed up this work by comparing the effects of intramuscular injections of 2 mg of atropine sulfate alone and mixed with 750 mg of P2S on heart rate and sweating in nine exercising men. These volunteers worked at 25°C and a relative humidity of 65-75% until their heart rates had risen to 110-130 beats/min and then got injections of atropine alone or of atropine and P2S. The exercise was continued for an additional period. The dose of atropine increased the mean heart rate at the end of the period of exercise by 34.4% and decreased the mean rate of sweat production by 36.1%. Addition of P2S to atropine had no important effect on these changes.

The General Practitioner Research Group (136) surveyed the effects of drugs in women in the United Kingdom during the first trimester of pregnancy. Of 661 pregnant women who took drugs of some sort during the first trimester, 57 (8.6%) had either miscarriages, stillbirths, neonatal deaths, or abnormal children. Antihistamines were involved in 38 of these, barbiturates in 10, and female sex hormones in 10. Antihistamines had been used by 76.9% of the pregnant women, barbiturates by 9.2%, and female sex hormones by 9.1%. Thus, women who used barbiturates or female sex hormones during the first trimester of pregnancy were the most likely to have one of the four types of reproductive accidents considered. A pregnancy that resulted in a blind infant had involved the use of scopolamine during the eighth week and of promethazine during the ninth week. Scopolamine was the only tropic acid ester used by a pregnant woman who delivered an abnormal child in this survey.

Hellman *et al.* (137) studied the reliability of the response of the fetal heart to administration of atropine to a pregnant woman, the fetal heartbeat being recorded and analyzed by a computer as an indicator of transplacental transfer. Intravenous injection of atropine sulfate at 22 ug/kg during a 2-min period to 28 normal pregnant women resulted first in slowing of fetal

heart rate and then tachycardia in 26 cases. In two cases, there was no apparent transfer of atropine to the fetus, although both women delivered apparently normal infants.

Mellin (138) studied the consequences of the use of drugs during the first trimester of pregnancy by 3,200 pregnant women seen at the Columbia-Presbyterian Medical Center in New York during 1953-1957. Of these 3,200 pregnancies, 266 were recorded as resulting in malformed infants. Two control groups were established by selecting 266 records immediately preceding and 266 immediately after each record relating to a malformed infant. Drugs were used during the first trimester by 53% of the women who delivered malformed babies, whereas $50.9\% \pm 1.9\%$ of the women in the control groups had used drugs during the first trimester. The difference is not significant. However, when fetal and neonatal deaths among the offspring of women who used drugs during the first trimester and of those who did not were summed separately, only 4.9% of the offspring of the latter group died in utero or soon after birth, whereas 9.5% of the offspring of the drug-users died before or soon after birth. Atropine was found to have been taken by one woman whose infant died soon after birth among 13 offspring of women who had used this drug during the critical period of embryogenesis; three of the other 12 babies of these women were malformed. Of three women who had taken scopolamine during the first trimester, two delivered apparently normal children. There is no way of determining from the paper what other drugs the women who used these two anticholinergic substances may have taken, except that one of seven babies with both congenital heart disease and another unspecified malformation was born to a woman who had used chlorpromazine, dimenhydrinate, meperidine, morphine, and secobarbital sodium in addition to scopolamine.

Hellman and Fillisti (139) extended the study by Hellman et al. (137) of placental transmission of atropine in normal women by studying 24 pre-eclamptic women, three with eclampsia, 11 with chronic hypertension, and 16 with diabetes. Sixteen of the 24 pre-eclamptic patients, two of the three eclamptic patients, and four of the 24 records on the 16 diabetic women gave no evidence of transfer of atropine to the fetus. Two of the negative records on diabetic mothers can be paired with one each from the same woman that provided evidence of transplacental transfer of atropine. Another of the negative records pertained to a woman who had yielded three records indicating placental transmission of atropine to the fetus. The general conclusion from both studies (137,139) was that the atropine test of placental function is not sufficiently precise to indicate whether placental function is normal in any given case.

Quilligan (140) pointed out that comparison of blood from the umbilical veins and arteries of placentas

of six women who received 1.0-1.5 mg of atropine and of 13 women who received no atropine revealed that the umbilical blood from the atropine-treated women had lower pO_2 s than that from the control patients, although the acid-base relationships of the umbilical blood from the two groups were not particularly different.

Kivalo and Saarikoski (141) studied transplacental transmission of atropine by injecting [3H]atropine intravenously (0.5 $\mu g/kg$) into 25 women undergoing delivery by Cesarean section. Samples of blood were taken from the antecubital vein of the mother and from the umbilical vein and artery. The investigators found that the concentration of the label in blood from the umbilical artery was about 50% that in blood from the umbilical vein. At 1 and 5 min after injection, the concentrations of 3H in the blood from the umbilical vein were 12% and 93%, respectively, of those in the maternal venous blood at the same times. Atropine seems to cross the placenta rapidly.

Onnen et al. (142) estimated the concentrations of atropine in blood samples by bioassay on isolated ileum from guinea pigs stimulated by acetylcholine. They gave rapid intravenous injections of atropine sulfate to 28 pregnant women at 12.5 $\mu g/kg$ and found that tachycardia in the fetus started 5-10 min after injection, at a time when the initial rapid decrease in the atropine concentration in the mother's blood had been replaced by a comparatively low rate of decrease. In another experiment, they gave 45 women in labor atropine injections and took samples of maternal and of mixed cord blood at birth. They found that 5-15 min after injection the ratio of the mean concentration of atropine in cord blood to that in maternal blood was 1.2 ± 0.5 . This paper also indicates that atropine is transferred across the placenta within a matter of a few minutes.

In addition to the effects on the reproductive functions of the female, there has been some study of the effects of tropic acid esters and related compounds on sexual function in the male. One paper on this topic (125) has been mentioned above. Horowitz and Goble (143) have reviewed the literature on drug-induced sexual dysfunction in the male and have concluded that any drug with atropine-like effects may interfere with penile erection. (They pointed out that impotence induced by antimuscarinic drugs may occur without reduction of libido and may thus be particularly frustrating to the male.)

Knuutila et al. (144) reported finding an unusually high incidence of chromosomal aberrations (broken chromatids, acentric fragments, dicentric chromosomes, etc.) in lymphocytes from a woman who had been vaccinated against rubella and 3 d later had received a variety of drugs, including atropine, in connection with an operation on varicose veins; after the operation, she had been given indomethacin for 10 d and furosemide for 12 d. The young woman was considered to be in good health. Lymphocytes

cultured from the blood of four other women who had been vaccinated against rubella also contained unusually large numbers of broken chromatids, but did not have the other chromosomal abnormalities. The percentages of metaphase cells with broken chromosomes from these four women were less (by 3-11%) than that in the blood from the first patient (15%). The investigators suggested that the major aberrations in the first patient's chromosomes may have resulted from a combined effect of the vaccine and one or more of the 10 drugs that she took during and after her operation. They recommended, therefore, that prescribing drugs should be avoided, if possible, near the time of vaccination for rubella. (In view of the fact that the investigators had no knowledge of the state of the patient's chromosomes before her vaccination and operation, their suggestion and recommendation seem to be on rather shaky ground.)

Minich *et al.* (145) attempted to detect mutagenic products in the urine of patients who were taking a large variety of drugs, including atropine, as a part of their therapeutic regimens in the hospital. The urine was tested with and without the addition of a preparation of microsomes from the livers of rats that had been given a 1% solution of phenobarbital in place of drinking water for 6 d before removal of the livers. Minich *et al.* found no evidence of mutagens in the urine of the patients given atropine.

HUMAN DATA FROM EDGEWOOD ARSENAL

Grob *et al.* (147) compared the effects of atropine administered intravenously and intramuscularly. Four subjects were used. Two received injections of 2 mg of atropine sulfate by the intramuscular route only; one of these subjects received only one injection, and the other, two. A third subject received 1 mg of atropine sulfate intravenously on one occasion, 1 mg of atropine sulfate intramuscularly on another occasion, and 2 mg of atropine sulfate intramuscularly on four other occasions. The fourth subject received 1 mg of atropine sulfate intravenously on two occasions and 2 mg of atropine sulfate intramuscularly on three occasions. Atropine sulfate by either route of administration induced an increase in heart rate, an increase in skin resistance, an increase in pupil size, dryness of the skin, and an increased sensation of dryness of the mouth. In general, the effects appeared earlier after intravenous than after intramuscular injection. Prior administration of sufficient TEPP to increase sweating and salivation and to induce anorexia and mild nausea slightly delayed the onset of the effects of atropine and slightly reduced the extent of those effects.

Marzulli and Cope (148) gave 40 men single injections of 1 mg of atropine intramuscularly, 28 men two doses of 1 mg of atropine 90 min apart, and 20 men single doses of 3 mg of atropine. The last group of subjects contained men who had received the two doses of 1 mg of atropine at least 2 wk previously and the single dose of 1 mg still earlier. The injections were into a deltoid muscle. Testing of the subjects began 30 min after each dose of 1 mg of atropine and 60 min after the dose of 3 mg. A battery of 10 tests, including nine

for grading visual acuity, stereopsis, and other properties of the eye and one for grading cognition, was used.

Four of 25 subjects who had received intramuscular injections of saline reported slight dryness of the mouth, two complained of headache, and one who had complained of headache also mentioned a slight numbness of the lips.

The 40 men given intramuscular injections of 1 mg of atropine all reported dryness of the mouth; 17 complained of dizziness, 13 of feeling tired and sleepy, 10 of dryness of the nose, and eight of having difficulty in reading.

The 28 men who received 2 mg of atropine all complained of dryness of the mouth; 17 complained of feeling tired and sleepy; 12 reported either that they had slept during the day or had gone to bed earlier than usual; nine complained of feeling incoordinated.

The 20 subjects given 3 mg of atropine all reported dryness of the mouth; 18 had felt tired or sleepy; 14 recalled either that they had slept during the day or had retired early in the evening; seven reported that their sleep during the night after the injection had been broken by vivid and disturbing dreams. Three men in the latter group reported seeing objects in unusual colors; a fourth had a visual aura. No true hallucinations were described.

The only significant objective changes seen in this study were a decrease in the ability of the eye to accommodate for near vision and tachycardia. The mean magnitude of accommodation by the eyes of the 20 men before they were exposed to atropine was 10.3 diopters (148). After a single dose of 1 mg, the mean magnitude of accommodation was 8.5 diopters. When the same subjects had been given two doses of 1 mg, the mean amount of dioptric change was 7.5. After a single dose of 3 mg, the eyes of the same 20 subjects had a mean magnitude of accommodation of 4.8 diopters.

This report provided no data to enable the reader to judge whether the tachycardia that followed the various doses of atropine was dose-related; data on heart rate are provided only after the single dose of 3 mg. The mean heart rate in the 20 men before exposure to atropine was 75.8 beats/min. At 15 min after injection, the mean was 116.8 beats/min. After another 15 min, the mean was 117.8 beats/min. Thereafter, the heart rate decreased gradually, the mean at 7-8 h after the injection being 66.1 beats/min. The mean was below that during the control period from the third hour to the eighth hour after injection.

Himwich (149) used four subjects to study the effects of intramuscular atropine at 0.07-0.12 mg/kg on the EEG, personality structure, heart rate, breathing rate, and pupil diameter. The pupil diameter increased by a mean of 114.3%, the heart rate by a mean of 51.0%, and the breathing rate by a mean (for only three subjects) of 48.5%. There was an increase in the voltage of the predominant frequency in the EEG, impairment of the alerting reaction on opening the eyes, and increased proportions of slow waves and spindles in the EEG, even though the subjects were awake and responsive to spoken commands. All four subjects found concentration on a single topic difficult, both attention and logical progression of

thought being attenuated. Judgment became poor, sensitivity to the environment was lessened, the grasp of reality was weakened, and drowsiness affected all four subjects. Three of the subjects became irritable, although the mood of the fourth was predominantly euphoric. The three irritable subjects had disturbed sleep and fantastic dreams during the night, whereas the fourth slept well. All four subjects had some degree of ataxia; two complained of urgency of urination and congestion of the conjunctivae. The most persistent effect of atropine noticed in this study was paralysis of accommodation, which disappeared gradually during 3-4 d after injection.

Wechsler and Koskoff (150) used nine surgical patients to study the effects of doses of atropine between 1.3 and 5.85 mg--administered by intravenous, intramuscular, or intracisternal injection--on cerebral blood flow and metabolism and on neural and cardiac indexes of normal function. One patient received atropine intracisternally on two occasions, 1.8 mg the first time and 4.2 mg the second time. Three patients were given intravenous doses of 1.3-1.6 mg of atropine, and five were given intramuscular doses of 2.0-5.85 mg. The intracisternal injections induced no changes in heart rate or blood pressure within 30 min after injection. The intravenous injections produced tachycardia in the three patients so treated and small increases in mean arterial blood pressure in two of them, whereas the third had a small decrease in blood pressure within 3 min after injection. All five patients given intramuscular atropine developed tachycardia, and four of them (the only ones whose blood pressures were recorded) developed small increases in mean arterial blood pressure. Atropine by any route of administration in these studies induced no consistent changes in cerebral blood flow, cerebral vascular resistance, cerebral consumption of oxygen, cerebral arteriovenous oxygen difference, cerebral respiratory quotient, or pH of blood from the jugular vein. The only significant neurologic change after administration of atropine was dilatation of the pupil. Only one patient given atropine yielded any indication of an effect on the EEG: a minimal depression of the alpha component. No morbidity was attributable to the atropine. The authors concluded that timidity in the use of atropine is not warranted.

Andrews et al. (151) used nine subjects in a preliminary comparison of the effects of atropine sulfate injected intramuscularly and subcutaneously in warm (35°C, 80% relative humidity) and cool (16°C, ambient relative humidity) environments and 24 subjects in a preliminary comparison of the effects of inhalation of 2 mg of atropine sulfate as a dust and as an aerosolized solution in water. Finally, 10 subjects were used to repeat the comparison of the effects of atropine sulfate administered by the two methods of injection in warm and cool environments and to compare the effects of 2 and 5 mg of atropine sulfate inhaled as a dust and an aerosolized aqueous solution in the warm environment. The report did not state whether the same subjects were used in more than one part of this study.

The results of the study indicated that 2 mg of atropine sulfate had practically identical effects on heart rate and sweating, whether injected intramuscularly or subcutaneously.

The mean increase in heart rate after injection of 2 mg of atropine sulfate in the warm environment was 42.7%, whereas that in the cool environment was 31.3%. The mean increase in heart rate after inhalation of 2 mg of atropine sulfate as a dust or an aerosolized solution was 23.7%; the mean increase after inhalation of 5 mg of the drug was 48.1%. The increases with the two types of dispersion of atropine sulfate were essentially identical for the two doses used. It is apparent that inhalation of atropine sulfate is not as effective a method of delivery, with heart rate as the indicator, as intramuscular or subcutaneous injection.

Although inhalations of 5 mg of atropine sulfate induced an increase in heart rate comparable with that induced by the injection of 2 mg, all subjects reported headaches, giddiness, tiredness, lethargy, and muscular weakness or incoordination after inhaling the 5-mg doses. It took 24 h for all the subjects to feel able to return to their usual activities after inhaling the larger dose of atropine sulfate, and several reported visual hallucinations, euphoria, and dysuria. The authors concluded that the use of inhalators for self-administration of atropine sulfate would be impractical and that intramuscular injection would be the easiest method to teach to nonmedical persons.

White et al. (152) gave 12 subjects intramuscular doses of atropine sulfate at 22.6-134.0 $\mu\text{g/kg}$ (mean, 50.8 $\mu\text{g/kg}$). The mean blood pressure before administration of atropine sulfate was 123/79; after administration, it was 129/91. The mean rate of the radial pulse was 69 beats/min before administration of atropine; within 30-60 s after injection, it was 61.5 beats/min; it increased later, reaching a mean maximum of 100.5 beats/min. The mean breathing rate before atropine was administered was 15.1/min; after atropine had been administered, it rose to 17.1/min. The mean diameter of the pupil was estimated to be 4.8 mm before administration of atropine; under the influence of that drug, it increased to 6.5 mm. Eight of the 12 subjects had extensions of the near point under the influence of atropine, the subjects given the two largest doses having the greatest increases.

All 12 subjects complained of dryness of the mouth; six became restless; two complained of sensations of heat on their skins, although they were in an air-conditioned room; 10 were observed to become drowsy; 10 found that walking a straight line was difficult; and five had malaise, headache, vertigo, and slight nausea. These complaints had disappeared by the following morning.

Other effects of atropine sulfate seen in the 12 subjects who took part in this experiment, in order of decreasing incidence, were sleepiness, shortened attention span and blurred vision, dreaming, eye irritation, lack of desire for mental work, and urinary urgency. The usual changes in the EEG pattern after administration of atropine were a flattening of the record, a decrease in frequency, and intermittent blocking of the alpha rhythm. There was a decrease in alerting on opening the eyes. Five subjects had notable improvement in photic driving after administration of atropine, and five others had slight improvement. Only one subject had a decrease in response to photic stimulation.

In attempting to devise a battery of tests useful in assessing the effects of chemicals on human capabilities, Elkin et al. (153) gave 11 subjects intramuscular injections of scopolamine at 12 $\mu\text{g}/\text{kg}$ after the subjects had spent a day gaining familiarity with the test battery. Four subjects received placebo injections. The only part of the test battery that may have been affected by the placebo was a manual-dexterity test in which the subject moved as many blocks as possible in 30 s from one hole to an adjoining hole. The number of blocks moved 0.75 and 2.25 h after the placebo was about 8.3% greater than the number moved before administration of the placebo and 4.25 h after the placebo. Scopolamine decreased both near and far visual acuities, the effect on near visual acuity being much the greater. In the manual-dexterity test, scopolamine decreased by 38.6% the number of blocks moved, this decrease persisting for 2.25 h after the injection of scopolamine and then diminishing gradually. By 8.75 h after the injection, the number of blocks moved was only slightly below the baseline measurement. Mean grip strength was lowered from about 54 kg to about 48 kg by the dose of scopolamine. Scopolamine lowered the mean duration of balancing on a beam on one foot from about 17 to about 5 s. The mean number of additions performed within 3 min decreased from about 42 to about four. The mean number of digits that could be recalled after auditory presentation decreased from about 7.1 to about 5.1. The mean ability to estimate 10 was altered only slightly by scopolamine in the direction of underestimation. However, the variance of this estimation was increased by about 128% by scopolamine. The mean reaction time (the subject pressed a switch as soon as possible after receiving a visual cue) increased from 0.22 to 0.38. The authors concluded that the most dramatic change was in near visual acuity. Grip strength, simple reaction time, and estimation of elapsed time were affected only slightly by scopolamine.

Kitzes et al. (154) analyzed the records of 18 subjects who had been given intramuscular injections of atropine at 32-125 $\mu\text{g}/\text{kg}$ and who weighed 57.2-90.0 kg. The results on the Number Facility Test (completion of as many additions as possible during 3 min) were used to grade each subject's response to the drug. No correlation between body size and decrease in the Number Facility Test was found.

Cummings and Craig (155) exposed 10 men to temperatures up to about 52°C to induce a range of rates of sweating and then subjected them to a variety of doses of atropine sulfate by intravenous injection of various priming doses followed by infusions of various concentrations of the drug for 13-53 min. The total dose of atropine received by a subject varied between zero and 1.6 mg; the report was unclear on whether this value is for atropine alkaloid or atropine sulfate. Higher rates of sweating, induced by exposure to high environmental temperatures, were found to require larger doses of atropine to inhibit sweating. Sweating recovered rapidly (6-74 min) after the end of infusion of atropine sulfate, particularly at the higher temperatures.

Kligman and Copelan (156) reported that 13 subjects had been exposed to atropine. No details or results of the study were given.

Crowell and Ketchum (157) used 33 normal male volunteers to assess the ability of physostigmine to antagonize the deliriant activity of scopolamine. The volunteers received intramuscular injections of scopolamine hydrobromide at 24 $\mu\text{g/kg}$; subgroups of the intoxicated men received physostigmine salicylate intramuscularly at 50 $\mu\text{g/kg}$ 15, 30, or 90 min after injection of scopolamine. Those treated 15 min after scopolamine did not have any immediate reversal of feelings of fatigue and drowsiness and gradually developed a typical delirium, but at a lower rate than those who had not been given physostigmine. Physostigmine lowered the heart rate in these men and maintained it on a plateau below that in the untreated group until the heart rate in the untreated group decreased to equal that in the treated group 3-4 h after the scopolamine had been administered. Physostigmine had only a minor and brief effect on the diameter of the pupil, but decreased significantly the decrement in the Number Facility Test due to the dose of scopolamine during the 2 h after its administration.

The subjects treated with physostigmine 30 min after the injection of scopolamine improved dramatically and rapidly in their performance of the Number Facility Test. These men relapsed into delirium about 3 h after administration of physostigmine and then recovered at about the same rate as the untreated men. The effects of physostigmine on heart rate and pupil diameter were similar to those in the group treated earlier in the intoxication.

The men treated 90 min after injection of scopolamine, after development of a full-blown intoxication, improved dramatically and rapidly, the change being noticeable within 10 min of injection of physostigmine and becoming maximal within 30 min. At that time, the subjects were alert, well-oriented, and logical. Their performance in the Number Facility Test decreased slightly about 1.5 h after the dose of physostigmine, but did not fall to near the level of performance of the untreated group at that time. The performances in the Number Facility Test of the untreated group and the group treated with physostigmine became essentially identical at about 6 h after the dose of scopolamine. The heart rate decreased within 30 min after the dose of physostigmine, but this drug had little effect on pupil size.

Baker *et al.* (158) summarized their studies with 82 volunteers given scopolamine. No details of the experiments or of their results were given, but the individual subjects were said to have reacted quite differently to a given dose of scopolamine. After extensive analysis of the data, the authors concluded that research was needed to identify the factors that cause "subject by treatment" interactions and that means for controlling for them were necessary for obtaining consistent effects by a given chemical.

Krieger (64) examined the effect of injected atropine on the circadian variation in the concentration of

17-hydroxysteroids in human plasma, using two healthy male subjects. One subject was given a subcutaneous injection of 3.0 mg of atropine, the other a similar injection of 3.5 mg. Three other subjects took oral doses of 2.5 mg of atropine. None of these subjects gave any evidence of an effect on the usual pattern of variation in plasma 17-hydroxysteroids. The author pointed out that the dose of atropine used in these human studies was only about one-tenth that used in experiments with cats in which atropine had been found to block the nocturnal rise in plasma 17-hydroxysteroid concentration, when administered before the time at which the circadian rise could be expected to occur.

Safer (159) investigated the possible interplay of sleep deprivation and scopolamine intoxication in producing deterioration of human abilities. Men were deprived of sleep for one or two nights and were then given scopolamine hydrobromide intravenously at 5 or 10 $\mu\text{g}/\text{kg}$. Behavior was rated with a checklist. The performances of the subjects in the Number Facility Test, a manual-dexterity test, and a vigilance test were evaluated. Eleven subjects were given scopolamine at 10 $\mu\text{g}/\text{kg}$, 10 were deprived of sleep for a night and given intravenous saline, six were deprived of sleep for a night and were given scopolamine at 10 $\mu\text{g}/\text{kg}$, two were given scopolamine at 5 $\mu\text{g}/\text{kg}$, four were given scopolamine at 5 $\mu\text{g}/\text{kg}$ after being deprived of sleep for a night, and four were given scopolamine at 5 $\mu\text{g}/\text{kg}$ after being deprived of sleep for two nights.

The combination of loss of sleep for one night and scopolamine at 10 $\mu\text{g}/\text{kg}$ had a more than additive effect on performance in the Number Facility Test and considerably more effect on manual dexterity than the scopolamine alone. The combination also produced hallucinations in about 2.7 times as many subjects as the same dose of scopolamine alone. The results with the lower dose of scopolamine were similar to those reported above, but less striking. The men deprived of sleep for two nights became so somnolent after the dose of scopolamine that the tests could not be run.

The effects of large intramuscular doses of atropine on the electrocardiogram were examined by Hayes *et al.* (160, 161), with six healthy male volunteers. The volunteers were given atropine sulfate at 175 $\mu\text{g}/\text{kg}$. By 2 min after injection, there was slowing of the heart without any striking change in the ECG record. At 5 min after the dose, the P wave was engulfed by the QRS complex, and an A-V nodal rhythm was instituted. This A-V dissociation was temporary, a sinus tachycardia becoming apparent at 6-7 min after the injection and persisting for about 3 h.

Four volunteers who had received scopolamine, three who had been given atropine, and nine who had received no chemicals were interviewed and examined at some unknown time after their service as experimental subjects (162). None of the subjects felt that he had suffered physical or psychic injury as a result of serving as a subject. Blood counts for all the former subjects were within the normal range. Three of the former control subjects and one former subject who had been

given both scopolamine and a salt of LSD had up to 10 white cells per high-power field in their urine; that suggested low-grade urinary tract infection. One former subject who had been given atropine had a mildly positive test for urinary glucose (there are so many possible causes of such a situation that no precise meaning can be attached to it). One of the former control subjects had an increased activity of GOT in his serum, and another had a slightly increased concentration of BUN. No abnormalities of serum chemistry were recorded for the former subjects who had been given atropine or scopolamine. This study revealed, therefore, no definite evidence of long-term physical or psychic injury by esters of tropic acid.

Klapper *et al.* (163) examined the records of eight subjects who had been given intramuscular injections of atropine at 62 $\mu\text{g/kg}$, six who had been given atropine at 104 $\mu\text{g/kg}$, and six who had been given scopolamine at 11.8 $\mu\text{g/kg}$. The performance of each subject on the Number Facility Test after injection was compared with his scores on the three validity scales and 10 standard scales of the Minnesota Multiphasic Personality Index (MMPI). The best correlations between performance in the Number Facility Test and scores on scales in the MMPI for the men given atropine at 62 $\mu\text{g/kg}$ were with those in the hypochondriasis and mania scales. The best correlations for the men given atropine at 104 $\mu\text{g/kg}$ were with scores in the lie and mania scales. For the men given scopolamine at 11.8 $\mu\text{g/kg}$, the best correlations were with scores in the hypochondriasis and lie scales. If atropine and scopolamine are taken as representative of the group of tropic acid esters, the best indexes of performance in the Number Facility Test under the influence of such esters are the scores in the lie, hypochondriasis, and mania scales of the MMPI. After a similar analysis for five anticholinergic compounds, three other substances with atropine and scopolamine, Klapper *et al.* (162) decided that scores in the positive test-taking attitude, hypochondriasis, and mania scales of the MMPI were positively related to performance in the Number Facility Test.

Sidell *et al.* (164) reported that intravenous injection of the potent cholinesterase inhibitor, S-(diisopropylaminoethyl)-ethylmethylphosphonothioate, at 1.5-1.7 $\mu\text{g/kg}$ into men 1.5 h after intramuscular injection of scopolamine hydrobromide at 24 $\mu\text{g/kg}$ was effective in reversing the performance and cognitive decrements produced by the scopolamine. If the anticholinesterase was administered 30 min after scopolamine, the initial therapeutic benefit was less than when it had been given at 90 min. This anticholinesterase had a more durable effect than physostigmine, its antagonistic activity persisting as long as the agonistic activity of scopolamine, whereas that of physostigmine had been found by others to last for only 2.5-3 h.

Ketchum *et al.* (24,165,166) reported the results of intramuscular injection of atropine sulfate at 32-175 $\mu\text{g/kg}$, of intravenous injection of scopolamine hydrobromide at 5-24 $\mu\text{g/kg}$, of intramuscular injection of scopolamine hydrobromide at 5-24 $\mu\text{g/kg}$, of intramuscular injection of scopolamine methylbromide at 5-30 $\mu\text{g/kg}$, and of attempts to find

antagonists of intoxication with these chemicals. The research involved 127 individual experiments. The ED₅₀s of atropine sulfate to initiate the following effects to recognizable extents were: poorly coordinated, 89 µg/kg; attention span shortened, 95 µg/kg; stumbling gait, 106 µg/kg; restlessness, 126 µg/kg; temporal confusion, 130 µg/kg; inability to obey simple orders, 135 µg/kg; and hallucination, 169 µg/kg. Although the ratios between the ED₅₀s of atropine and of the other anticholinergic compounds for the production of the different effects varied somewhat, intravenously injected scopolamine hydrobromide was about 8.7 times as active as atropine sulfate and, when injected intramuscularly, about 7.5 times as effective as atropine sulfate. Scopolamine methylbromide was about 4 times as active as atropine sulfate, except that no dose of the former substance produced as much as a 90% decrease in performance in the Number Facility Test, so comparative potencies had to be assigned on the basis of less than maximal actions by atropine and scopolamine. Therefore, scopolamine methylbromide was only about 53% as potent as the hydrobromide.

Intramuscular injections of scopolamine hydrobromide at 24 µg/kg and of atropine sulfate at 175 µg/kg induced approximately equal decrements in performance in the Number Facility Test, except that the maximal effect after scopolamine was attained in about half the time required to reach that after atropine. Intravenously injected scopolamine hydrobromide produced its maximal effect even more rapidly than that injected intramuscularly. Scopolamine methylbromide was slower in exerting its maximal tachycardial effect than scopolamine hydrobromide, but no information about the rapidity of its action on performance in the Number Facility Test was provided in any of the three reports (24,165,166). The increase in heart rate induced by scopolamine methylbromide was greater than those induced by the same amounts of scopolamine hydrobromide or atropine sulfate.

The decreases in performance in the Number Facility Test induced by atropine or scopolamine were antagonized rapidly by intramuscular injections of physostigmine at 15-60 µg/kg, but the antagonistic effect wore off after 3-4 h. Neostigmine had no immediate effect on performance in the Number Facility Test, but may have increased slightly the rate of spontaneous recovery 5-10 h after the intramuscular dose of atropine sulfate. With respect to the tachycardia induced by atropine, neostigmine may have exerted a prompter and more potent bradycardiac action than physostigmine. Physostigmine also antagonized the decrement in performance on the Number Facility Test induced by scopolamine. The organophosphorus anticholinesterase compounds, DFP and Sarin, had some antagonistic effect against the decrement in performance in the Number Facility Test induced by scopolamine, but these effects were less striking, although more persistent, than those of physostigmine. Tetrahydroaminoacridine, also an inhibitor of cholinesterases, had some antagonistic activity against the decrease in performance in the Number Facility Test due to scopolamine. Intramuscular injection of chlorpromazine after a

small dose of scopolamine hydrobromide (8 μ g/kg) increased the decrement in performance in the Number Facility Test due to the scopolamine. When 50 mg of chlorpromazine was injected intramuscularly after intramuscular scopolamine hydrobromide at 8 μ g/kg, the resulting decrease in the score in the Number Facility Test approached that induced by scopolamine hydrobromide at 24 μ g/kg alone, although chlorpromazine by itself induced only a slight decrease in performance. Perphenazine, another derivative of phenothiazine, had effects similar to those of chlorpromazine.

Sidell (167) reported that absorption of atropine from an intramuscular site of injection in man was prolonged when it was mixed with a 30% solution, but not when mixed with an 18% solution, of pralidoxime chloride. Because absorption of atropine was also delayed when it was mixed with an 8.5% solution of sodium chloride, Sidell suggested that the delayed absorption with 30% pralidoxime chloride may have been due to osmotic effects, rather than to any specific effect of the oxime. However, as pointed out by Sidell, although absorption of atropine from the mixture with 8.5% sodium chloride was delayed, eventually this mixture caused the same mean increase in heart rate in the six volunteer subjects as atropine alone at the same time after injection. In contrast, neither mixture of atropine with pralidoxime chloride accomplished this; the mixture of atropine with the 18% solution came closer than the mixture with the 30% solution to achieving the same increase in mean heart rate as atropine alone. Therefore, pralidoxime may have had a dose-related effect on the total absorption of atropine. Whether this is due to a simple osmotic effect on the absorption of atropine, with some of the alkaloid held at the site of injection by unabsorbed pralidoxime chloride in solution, or to some more specific effect of the oxime is not clear.

Sidell (168) studied the effectiveness of intravenous administration of physostigmine as an antagonist of the central (Number Facility Test) and peripheral (heart rate) actions of atropine and scopolamine. The report simply reiterated the effectiveness of physostigmine as an antagonist of the central actions of these two alkaloids and demonstrated that repetition of administration of physostigmine after disappearance of the effect of a first dose renewed the antagonism of persistent central actions of the alkaloids, but, like the first dose of physostigmine, had only a minor effect on the tachycardia due to the alkaloids.

SUMMARY

This review of the general literature portrays the tropic acid esters with which this report is concerned as compounds that are not particularly lethal in single or repeated doses. The members of the group that are tertiary amines may affect psychic activity in comparatively small doses, but there is fairly rapid adaptation to repeated doses. Removal of the compounds from the body, so far as it has been studied, seems to be comparatively rapid and to occur by a combination of urinary and fecal excretion and metabolic transformation. The

transport of intact atropine through the tubular epithelium of the chicken is by the cation transport mechanism and is reduced by drugs and chemicals that inhibit that transport process: quinine, choline, cocaine, and cyanine dyes (146).

Symptoms and signs of the action of the tropic acid esters on human subjects include complaints of mouth dryness and decreased secretion of saliva; increase in heart rate, possibly preceded by a short period of decrease in heart rate; dryness and flushing of the skin, particularly in the "blush area"; difficult urination; ataxia; disorientation; hallucination; delirium; dilated pupils, possibly nonreactive to light; prostration; and coma.

The greatest hazard to life arises from suppression of the ability to secrete sweat, which can give rise to fatal hyperthermia if body temperature is not controlled artificially during hot weather or strenuous activity. Another source of hazard to life arises from the effect of the compounds other than scopolamine and its quaternary form in accelerating heart rate and facilitating intramural conduction and transmission of impulses. These actions may result in serious arrhythmias up to and including ventricular fibrillation. The quaternary amine forms of the tropic acid esters in which we are interested are more active in some respects than the tertiary amines, as far as peripheral actions are concerned. This is especially true with respect to actions on nicotinic effectors in ganglia and striated muscles. The quaternary amines penetrate into the central nervous system poorly, but, once there, affect muscarinic effectors in the same way as the tertiary amines.

The anticholinergic compounds that are esters of tropic acid are not known to have any important mutagenic, teratogenic, or carcinogenic actions. There has been little study of long-term toxicity of these compounds, however, so one cannot say categorically that they have no carcinogenic activity. The rapidity with which atropine is removed from the body, although it cannot be taken as proof of noncarcinogenicity, does militate against a high probability of such an action by this compound.

ESTERS OF BENZILIC ACID

The master file of the Board on Toxicology and Environmental Health Hazards lists four compounds that are esters of benzilic acid. One of these compounds seems to be an isomer of another--both are benzilic acid esters of tropine. Of the 12 reports that concern administration of compounds of this group to volunteers, either at Edgewood Arsenal or at contractors' facilities, 11 are related to the results of studies with quinuclidinyl benzilate and three with tropinyl benzilate (these three reports are concerned exclusively with the L isomer of the ester). Accordingly, the literature review in this section centers on these two compounds insofar as possible, and especially on quinuclidinyl benzilate.

The first compound made in this group of esters of benzilic acid seems to have been N-diethylaminoethyl benzilate (benactyzine), whose synthesis was reported in 1938 by

Horenstein and Pahlicke (169). The synthesis of tropinyl benzilate was described in 1952 by Hromatka et al. (170). In 1952, Sternbach and Kaiser (171) reported methods for producing several bicyclic alcohols, including 3-quinuclidinol. These alcohols were then used to produce esters with organic acids (172), including benzoic acid. The esters were examined for their ability to induce relaxation of isolated rabbit intestine that was made to contract by acetylcholine; the esters of 3-quinuclidinol were found to have greater spasmolytic activity than the corresponding esters of diethylaminoethanol and other bicyclic alcohols. The 3-quinuclidinyl ester of diphenyl acetic acid was prepared in its L and D forms. The levorotatory ester was found to have most of the activity, the dextrorotatory ester having only very slight activity. The toxicities of the isomers were stated to have been identical, but no data on toxicity were reported. A paper by Randall et al. (173) gave the intravenous LD₅₀s for the mouse of the L isomer as 29.5 mg/kg and of the D isomer as 27 mg/kg.

The synthesis of esters of 2-tropanol was described by Archer and Bell (174) in 1961. In 1971, Atkinson and McRitchie (175) reported methods for synthesizing all four of the isomers of 2-tropanol. The chemical literature seems not to reveal when the production of L-2-tropanyl benzilate actually occurred, but it was probably in 1971-1972.

Randall et al. (173) assessed the potencies of 3-quinuclidinyl benzilate hydrochloride and N-methyl-3-quinuclidinium benzilate bromide in several different activities. The LD₅₀s of 3-quinuclidinyl benzilate for the mouse were about 1.46 and 2.04 times those of the quaternized compound after intravenous and intraperitoneal injection, respectively. In producing relaxation of isolated intestinal smooth muscle stimulated by acetylcholine, 3-quinuclidinyl benzilate was somewhat more effective than atropine. The 3-quinuclidinyl benzilate had some activity in relaxing spasm of intestinal smooth muscle induced by histamine and was quite effective in antagonizing that induced by barium. It was also effective in preventing contraction by bronchial smooth muscle on exposure to aerosolized methacholine and in overcoming the bradycardia and hypotension induced by injection of acetylcholine or by electric stimulation of the vagal nerves. A dose of 2 mg/kg prevented the response of the cat nictitating membrane to electric stimulation of the cervical sympathetic nerve.

The chemist who made 3-quinuclidinyl benzilate took about 0.5 mg of the compound and experienced not only dryness of the mouth and mydriasis, but also a sensation of weakness in the knees. Despite having received several subcutaneous doses of neostigmine, he slept fitfully that night and spoke incoherently during waking periods. On the next morning, he was still unsteady on his feet, and his pupils were dilated. By the second morning after he had taken the compound, all symptoms except those due to persistent mydriasis had disappeared. He revealed that he had felt confused and that time had seemed to pass very slowly. Two other employees involved in work with 3-quinuclidinyl benzilate had similar illnesses, with apparent hallucination in one patient (176).

In addition to those three occupational exposures, two of six patients given 0.1 mg of 3-quinuclidinyl benzilate three times a day as a treatment for parkinsonism had nocturnal confusion (176).

STUDIES IN ANIMALS

In an attempt to explain these confusional states, Schallek and Smith (177) studied the effects of five spasmolytic substances, including 3-quinuclidinyl benzilate and its quaternized form, on the electroencephalogram, the electrocardiogram, and the blood pressure of the dog. Intravenous injection of 3-quinuclidinyl benzilate at 0.01 mg/kg decreased the dominant frequency of the EEG of two of five dogs; a dose of 0.1 mg/kg enlarged to three the number of dogs in which the dominant frequency of the EEG was decreased. Larger doses, up to 10 mg/kg, did not further enlarge the proportion of dogs with a decreased dominant frequency. The dose of 0.1 mg/kg induced bradycardia in one of five dogs. A dose of 10 mg/kg induced bradycardia in all five dogs and resulted in death due to cardiac arrest in two of five.

The only dose of quaternized 3-quinuclidinyl benzilate that induced any alteration in the EEG was 10 mg/kg, which resulted in a decrease in the dominant frequency in the EEG of one of five dogs. This dose also induced bradycardia in three of five animals and hypotension in all five. No dogs given this dose of N-methyl-3-quinuclidinyl benzilate bromide died. Intravenous doses of 0.1 and 1.0 mg/kg caused tachycardia in one of five and two of five dogs, respectively.

Before proceeding with a discussion of the effects of 3-quinuclidinyl benzilate, it may be worth while to look at the available information on the lethality of single doses of the benzilic acid esters with which we are concerned. Table I-2 is based on information supplied by Hoffman-LaRoche, Inc., the Sterling-Winthrop Research Institute, and a number of investigators (36,173,176,178-189).

The benzilic acid esters of both diethylaminoethanol (179, 180,185) and tropine (182,183) were found to be capable of overcoming spasms of smooth muscle induced by acetylcholine and of inducing relaxation of smooth muscle, such as the circular muscle of the iris. Both compounds had some local anesthetic activity, both produced changes in the EEGs of experimental animals similar to those induced by atropine, and both had slight antihistaminic activity. Lisunkin (187) reported that tropinyl benzilate increased the effectiveness of other antispasmodic drugs, such as tropinyl diphenylacetate (tropacine), in antagonizing the tremor induced by subcutaneous nicotine.

Larsen (181) fed rats diets containing either 0.01% or 0.05% of the benzilic acid ester of diethylaminoethanol from 28 d of age until spontaneous death. Another group of rats was fed the basal diet. The rats fed benzilic acid ester gained weight slightly faster than those fed the normal diet, but died younger than the controls (means of 24 and 26 mo, compared with one of over 29.5 mo). No striking pathemas in the animals due to the benzilic acid ester were reported.

3-QUINUCLIDINYL BENZILATE (BZ, EA 2277)

McNamara (184) reported that rats placed into atmospheres containing 3-quinuclidinyl benzilate dispersed as a dust, as a thermally generated smoke, or as an aerosol of a 1% solution of the benzilic acid ester in methylene dichloride for concentration-time (Ct) products up to 29,508 mg.min/m³ experienced no permanent effects from the exposures, except occasional deaths randomly distributed with respect to intensity of exposure. One of four guinea pigs exposed to a Ct product of 10,123 mg.min/m³ and two of four guinea pigs exposed to a Ct product of 14,748 mg.min/m³ died. Mice exposed to an aerosolized 1% solution of the benzilic acid ester in methylene dichloride at Ct products up to 14,137 mg.min/m³ did not experience any clearly dose-related mortality, but 69 of 70 mice exposed to thermally generated smoke of 3-quinuclidinyl benzilate at Ct products of 8,213-9,331 mg.min/m³ died.

McNamara (184) reported that daily subcutaneous doses of 3-quinuclidinyl benzilate at up to 150 µg/kg on 5 days of each week for 3 wk induced no evident signs of toxicity in mice and guinea pigs. Dogs given daily intravenous doses of 100 µg/kg for 14 consecutive days had the same LD₅₀ as dogs that had not been pretreated, but the time between injection of the daily dose and the appearance of ataxia increased from 4 min to 14 min during the period of pretreatment. In dogs trained in a conditioned-escape routine and then given graded intravenous doses of 3-quinuclidinyl benzilate at up to 12.5 µg/kg, there was no effect on performance. A dose of 25 µg/kg resulted in failure to escape by four of four dogs.

The same report (184) summarized data on the effect of intraperitoneally injected 3-quinuclidinyl benzilate on spontaneous motor activity of the rat. When the data were plotted and the curve extrapolated to zero change in spontaneous activity, the maximal dose that could be given without altering spontaneous activity seemed to be about 0.12 mg/kg. No rats were given daily injections of saline, however, so a decision on what portion of the observed changes was attributable to the compound, rather than to the procedure, is not possible.

Studies of the absorption of 3-quinuclidinyl benzilate from solutions applied in a mixture of alcohol and cresol to the clipped skin of five species of animals, summarized in the same report (184), found that doses of up to 500 µg/kg produced ataxia in the cat after about 30 min and in the dog after about 55 min. No ataxia was produced in the goat or the monkey (probably rhesus) by the highest dose; the highest dose applied to the skin of the rabbit was 100 µg/kg. Doses of 50 µg/kg applied to the skin of the rabbit, of 100 µg/kg applied to the skin of the cat, of 250 µg/kg applied to the skin of the monkey, and of 500 µg/kg applied to the skin of the dog and the goat were the lowest doses found to have effects on the eyes. In all species except the monkey, recovery from the ocular effects was stated to occur overnight. In the monkey, the ocular effects apparently persisted for 2 d or more.

In the dog, an intravenous dose of 10 µg/kg produced a mean increase in four dogs of about 143% in heart rate, which decreased slowly during an observation period of up to 6 h, but was still evident at the end of that period. By 24 h after the dose, the heart rate was back to normal. A dose of 12.5 µg/kg, but not one of half that amount, decreased by 20% the mean time during which dogs would run on a treadmill. None of the experiments summarized in this report (184) seems to have involved any persistent effect of exposure to 3-quinuclidinyl benzilate in surviving animals. Neither did the report of Wal (186) mention any consistent toxic effects of this compound.

Ketchum *et al.* (189) reported that rats and dogs with whole-body exposures and rabbits and monkeys with head-only exposures to clouds of 3-quinuclidinyl benzilate dispersed in the field at wind speeds of 8-11 mph developed such effects as ataxia, dyspnea, mydriasis, cycloplegia, tachycardia, sedation, hyperactivity, and nasal stuffiness. The most persistent of these effects was cycloplegia, which persisted in three of five monkeys for more than 7 d. Most other effects disappeared within 48 h after exposures.

No study of long-term administration of 3-quinuclidinyl benzilate to experimental animals has been found. The closest approaches to such a study are 1-yr studies of the results of feeding the methyl bromide of 3-quinuclidinyl benzilate to rats in the diet (190) and of gavaging dogs 5 d/wk with the same quaternary amine salt of 3-quinuclidinyl benzilate (191). Rats were fed diets containing the quaternary amine salt at concentrations calculated to yield daily doses of zero, 5, 25, and 50 mg/kg. Dogs were gavaged with the quaternary salt at zero, 1, 5, and 25 mg/kg. In both experiments, no evidence of dose-related toxicity was seen. The livers of the male dogs that had received daily doses of 5 and 25 mg/kg seemed to be larger than those of the controls (3.0% and 3.2% of body weight, respectively, versus 2.7% for the controls), but were normal in structure on necropsy and in function (glutamic-pyruvic transaminase and alkaline phosphatase activities in serum and Bromsulphalein retention within normal limits). Although the compound used in these two studies was not 3-quinuclidinyl benzilate and probably was absorbed from the intestinal tract less rapidly and less completely than that compound, it contained the same combinations and arrangements of atoms. Therefore, the negative findings with respect to organ and tissue damage in the two studies may indicate that similar experiments with 3-quinuclidinyl benzilate would result in no chemically induced pathemas.

The great avidity of mitochondria from rat brain for 3-quinuclidinyl benzilate has been mentioned previously (56). Larsson *et al.* (192) recorded the infrared spectra of seven esters of benzoic acid, of eight esters of 3-quinuclidinol, and of atropine and scopolamine and attempted to correlate the relative strengths of the intramolecular hydrogen bond with the threshold doses of the various compounds in producing psychotomimetic effects in dogs. The data of Albanus (42) on the psychotomimetic activities of anticholinergic compounds were used for the comparison. Of the esters of benzoic acid,

the most active was 3-quinuclidinyl benzilate. According to Albanus's data, it was 50 times as active as tropinyl benzilate. Four other esters of 3-quinuclidinol were as active as 3-quinuclidinyl benzilate, but none was more active. Larsson *et al.* were not able to detect any correlation between the strength of the hydrogen bond, as derived from their spectra, and the biologic activities of the compounds estimated by Albanus.

Dogs given subcutaneous doses of tropinyl benzilate at 0.50 mg/kg became ataxic after a mean elapsed time of 16 min and obstinately progressive after a mean of 19 min; ataxia disappeared after a mean elapsed time greater than 283 min, whereas obstinate progression lasted only a mean of 244 min. Dogs given doses of 3-quinuclidinyl benzilate at 50 µg/kg became ataxic after a mean of 36 min and became obstinately progressive after a mean of 42 min. These two effects disappeared at mean times of greater than 313 and greater than 305 min, respectively. Tropinyl benzilate in a dose 10 times that of 3-quinuclidinyl benzilate produced toxic effects somewhat more rapidly than the latter, but these effects were less durable than those of the smaller dose of 3-quinuclidinyl benzilate.

Lavallee (50) reported that doses of 3-quinuclidinyl benzilate at 10 and 18 µg/kg had no progressive effects on performance of a multiple-stimulus conditioned-avoidance response by dogs, but doses of 24 and 32 µg/kg decreased the number of correct responses progressively. Monkeys given doses of 32 and 56 µg/kg may have made a few more errors in a visual-discrimination avoidance task than monkeys given no compound, but the difference was not statistically significant in the absence of control data.

Cats given intravenously injections of 3-quinuclidinyl benzilate labeled with ³H in the 3-position of quinuclidinol were used to determine the distribution of the label in the brain and brain stem (193). Taking 304 counts/min.mg in the nervous tissue as the halfway point between the highest and the lowest concentrations measured, the areas of the brain that retained the greatest concentrations of the label were motor cortex, sensory cortex, caudate nucleus, lateral geniculate, and medial geniculate (167, 140, 121, 107, and 101 counts/min.mg, respectively). The areas that retained smaller concentrations, in order of decreasing concentration of the label, were thalamus, hippocampus, hypothalamus, medulla oblongata, colliculi, cerebellar cortex, pyramids of the medulla, cerebral white matter, and cerebellar white matter.

Both cholinomimetic and anticholinergic compounds were found (193) to reduce the retention of the label in some areas of the brain, the most strongly affected areas varying with the compound. The cholinomimetic compounds used were tetrahydroaminoacridine (THA) and S-diethylaminoethyldiethylphosphorothioate (TETRAM), both inhibitors of cholinesterases. The anticholinergic compounds used were N-methyl-4-piperidinyl- (phenylcyclopentyl)-glycolate (EA 3443) and 302,028 (not identified except as an analogue of 3-quinuclidinyl benzilate). The last compound had a much more

general action than any of the others used, displacing the label of 3-quinuclidinyl benzilate from most of the sampled areas of brain and brain stem. In the cases of both EA 3443 and 302,028, displacement of the label was related probably to the substitution of one intoxicant by another, so that neither of these two anticholinergic substances would be practical antagonists of intoxication induced by 3-quinuclidinyl benzilate. Antagonism might reasonably be expected from THA, which partly displaced the label from motor cortex, lateral geniculate, medulla, and cerebellar cortex; antidotal activity by TETRAM--which displaced the label from lateral geniculate, thalamus, and hippocampus--is less likely. No attempts to assess antidotal effectiveness were made in this study.

Zvirblis and Kondritzer (56) found that a mitochondrial fraction prepared from rat brain adsorbed 3-4 times as much 3-quinuclidinyl benzilate as atropine in the physiologic range of pH. The optimal pH for adsorption of 3-quinuclidinyl benzilate was about 8.0, whereas that for atropine was about 9.7; at these pHs, the adsorption of the two anticholinergic compounds was about the same. THA decreased the adsorption of the benzilate by mitochondria, but a ratio of 100:1 (THA:3-quinuclidinyl benzilate) was required to reduce the adsorption by 50%. Both Ca^{2+} and PO_4^{2-} decreased the mitochondrial adsorption of 3-quinuclidinyl benzilate, the effect of Ca^{2+} not being particularly large and requiring an estimated ratio of 30,000:1 (Ca^{2+} :3-quinuclidinyl benzilate) to decrease adsorption of the benzilate by 50%. With phosphate, a ratio of about 21.5:1 (PO_4^{2-} :3-quinuclidinyl benzilate) would be expected to decrease adsorption of the benzilate by 50%.

Yamamura *et al.* (194) studied the adsorption of ^3H on particles in homogenates of various regions of monkey brain after incubation with [^3H]3-quinuclidinyl benzilate in solution in phosphate buffer at a pH of 7.4. The regions that were the most avid adsorbers of the benzilate, in order of decreasing uptake, were putamen, caudate nucleus, occipital cortex, cingulate gyrus, postcentral gyrus, hippocampus, amygdala, precentral gyrus, pyriform cortex, frontal cortex, superior colliculi, thalamus, and inferior colliculi. This distribution of adsorptive sites among various parts of monkey brain differs from that for uptake of ^3H from intravenously injected [^3H]3-quinuclidinyl benzilate by cat brain (193). The two studies are in agreement that the caudate nucleus is one of the most important areas of localization of the label in the form of [^3H]3-quinuclidinyl benzilate. Yamamura *et al.* found that uptake of the label by various areas of brain had a tendency to be correlated with choline uptake and with choline acetylase activity.

Yamamura and Snyder (195) found that interruption of the septal hippocampal tract, and elimination thereby of cholinergic afferents to the hippocampus, reduced by about 70% the activity of choline acetylase in homogenates of the hippocampus, but did not alter the binding of [^3H]3-quinuclidinyl benzilate by the particles of such homogenates. They proposed, as a possible explanation, that

presynaptic muscarinic sites that are innervated by the septal hippocampal nerve fibers do not adsorb the labeled benzilate, but that postsynaptic muscarinic sites just across the synaptic gap from the presynaptic ones do adsorb it. Another possibility mentioned is that there may be no presynaptic muscarinic sites in the hippocampus.

The ATPase in microsomes prepared from whole rat brain, from rat cerebral cortex, and from cerebral cortex of rats that had received 3-quinuclidinyl benzilate intraperitoneally at 1 mg/kg an hour before they were killed was found (196) to be enzymatically competent in all cases and not to be inhibited significantly by the addition of 3-quinuclidinyl benzilate in vitro. Furthermore, slices of guinea pig brain cortex incubated in vitro with 10^{-4} M 3-quinuclidinyl benzilate had the same patterns of loss of K^+ and uptake of Na^+ as slices incubated without the benzilate. Inasmuch as ATPase is related intimately to the active transport of the monovalent cation in nervous tissue, the experiment with the brain slices gave an additional indication that ATPase activity is not altered by 3-quinuclidinyl benzilate.

Jovic and Zupanc (197) measured the effects of 3-quinuclidinyl benzilate on oxygen consumption by rats and oxygen uptake by slices of rat cerebral cortex and medulla oblongata. Subcutaneous injections of the benzilate at 4-15 mg/kg decreased oxygen consumption by a mean of 12.1%; there was no clear dose-effect relationship. Rats whose oxygen consumption had been increased by a mean of 33.5% by a small dose of soman (25 ug injected subcutaneously) had the increase limited to about 2% by a subcutaneous dose of 3-quinuclidinyl benzilate at 15 mg/kg.

Slices of cerebral cortex and medulla oblongata stimulated by KCl at a final concentration of 100 mM increased their oxygen uptake by respective means of 59.2% and 41.7% (197); inclusion of 5×10^{-4} M 3-quinuclidinyl benzilate in the medium bathing the slices reduced the stimulated oxygen uptake to 16.3% for cerebral cortex and to 3.6% for medulla oblongata. When rats were given 3-quinuclidinyl benzilate subcutaneously at 5-20 mg/kg and were killed 1 h later for preparation of slices of cortex and medulla oblongata, there were dose-related decreases in oxygen uptake by both tissues stimulated by inclusion of KCl in the fluid surrounding the slices.

Frances and Jacob (81) reported that intraperitoneal injections of 3-quinuclidinyl benzilate into mice at 0.3-3.0 mg/kg decreased the concentration of acetylcholine that could be measured in the brain. There was a linear relation between the decrease in acetylcholine concentration and the logarithm of the dose of benzilate. The line for this relationship with 3-quinuclidinyl benzilate was close to and nearly parallel with that with scopolamine.

Aquilonius et al. (77) found that intravenous 3-quinuclidinyl benzilate at 50 μ g/kg produced about the same loss of acetylcholine from the rat cerebral cortex as atropine at 750 μ g/kg. Intravenous 3-quinuclidinyl benzilate at 0.15, 0.2, and 0.5 mg/kg produced greater initial increases in rat

locomotor activity than atropine at 1.3, 2, and 10 mg/kg. The immediate responses to the benzilate were not so well maintained as those to atropine. As the benzilate dose was increased from 0.1 to 0.2 mg/kg, locomotor activity increased rapidly to an almost maximal value that was about 9.3 times the control value.

The concentration of acetylcholine in rat hippocampal tissue was increased slightly (10.3%) by intraperitoneal 3-quinuclidinyl benzilate at 0.1 mg/kg (79); after a dose of 5 mg/kg, the acetylcholine concentration was reduced by 44.5%, compared with the mean concentration found in control rats. When septal lesions were created, the mean concentration of acetylcholine in the hippocampus increased by about 47.4%. As in intact rats, administration of 3-quinuclidinyl benzilate at 0.1 mg/kg increased the mean concentration of acetylcholine in the hippocampus, in this case by 5.3%. Unlike the result in intact rats, administration of 5 mg/kg resulted in a further increase in the mean concentration of acetylcholine in the hippocampus, to 16.6% above that in control rats. This last result was the converse of that found with atropine, but was similar to that found with scopolamine.

Meyerhöffer (198) found that L-3-quinuclidinyl benzilate was about 20 times as active as the Disomer in dogs in producing ataxia, tachycardia, and obstinate progression and in reducing salivation.

Kabes *et al.* (199) reported that rats given doses of 0.8, 2.0, and 5.0 mg/kg of 3-quinuclidinyl benzilate (by an unidentified route) had dose-related increases in spontaneous motor activity. Physostigmine (0.2 mg/kg), amobarbital (20 mg/kg), and chlorpromazine (2 mg/kg), all given by unidentified routes before the benzilate, held the spontaneous motor activity even below the control values.

Kabes (200) examined the effects of 3-quinuclidinyl benzilate on the abilities of rats to learn a conditioned-escape task and, once trained, on performance of the task. Doses of 0.5, 1, and 2 mg/kg of the benzilate hastened acquisition of the conditioned response in a dose-related manner, but a dose of 1 mg/kg had different effects on performance of the task in relation to the proficiency of the animal in its performance before the benzilate was administered. Rats that were poor performers (more than 20% errors in control sessions) and some that were good performers (less than 20% errors in control sessions) had their performance improved by the benzilate, whereas other good performers had their performances worsened.

Kabes (201) examined the effects of 3-quinuclidinyl benzilate on beagles. There were dose-related responses to doses of 10 and 50 µg/kg in autonomic functions (heart rate, pupil diameter, flow of saliva, etc.), motor functions (motor coordination, tremor, ataxia, etc.), and central nervous system functions (alertness, orientation, reaction to external stimuli, etc.). Physostigmine (1 mg/kg) aborted all these types of action. Routes of administration were not stated; physostigmine was said to have been injected, but the site was not stated.

Fusek *et al.* (202) used isolated rat jejunum and atria to examine the effects of 3-quinuclidinyl benzilate on tissue response to cholinergic agonists. The benzilate alone had no effect on isolated jejunum, but, when applied before a stimulant concentration of furtrethonium (10^{-7} - 10^{-4} M), it shifted the dose-response curve for furtrethonium to the right, i.e., a higher concentration of furtrethonium was required to produce a given response in the presence of 3-quinuclidinyl benzilate than in its absence.

The application of 3-quinuclidinyl benzilate at a range of concentrations (3 - 30×10^{-9} M) to isolated atria had no effect on the contractions of either spontaneously beating or electrically driven atria (202). When the atria were subjected to the influences of both furtrethonium and 3-quinuclidinyl benzilate, the dose-effect curve for the former was shifted to the right. This was similar to the result with isolated jejunum, the molar ratio of furtrethonium to 3-quinuclidinyl benzilate being about $3.3 \times 10^6:1$.

Herink *et al.* (82) injected 3-quinuclidinyl benzilate at 1 mg/kg into the peritoneal cavities of normal rats and rats with electrolytic septal lesions. In normal rats, there was no significant effect on aggressiveness, but defecation was decreased. In the rats with septal lesions, both aggressiveness and defecation were reduced by 3-quinuclidinyl benzilate (the latter being affected to the greater extent).

Poel (203) trained female rats to run in a circular runway for a reward of a drop of chocolate paste. After completion of training, the rats were given intraperitoneal injections of 3-quinuclidinyl benzilate at 0.1, 0.3, 1, 3, or 10 mg/kg and were placed in the runway 20 min later. The benzilate induced a marked increase in the time required for the rat to leave one reward area and begin to seek a second reward (latency); the increase in latency was dose-related. There was a dose-related increase in the time required for the rat to traverse the circular runway (running time), but the slope of the regression line for the relationship of running time to the dose of 3-quinuclidinyl benzilate was much smaller than that for the relationship of latency to dose. These results were interpreted as indicating that the benzilate intensified a conflict between a desire to remain in the vicinity of a prior reward and a desire to obtain a later reward, coupled with some interference with motor function similar to that found by Meyerhoffer (198) and Kabes *et al.* (199,200).

Bell and Gershon (86) found that intravenous injection of 3-quinuclidinyl benzilate at 50 ug/kg into dogs induced intoxication that persisted for more than 5 h. Intravenous injection of 1,2,3,4-tetrahydro-9-aminoacridine (Tacrine or THA) at 1 mg/kg within 30-50 min after administration of the benzilate induced recovery in only two of nine dogs, but similar treatment 2-3 h after the benzilate had been injected was successfully antidotal in seven of seven dogs. Yohimbine, which has cholinomimetic and sympatholytic actions (204), injected intravenously at 0.5 mg/kg within 30-50 min after the benzilate had antagonistic activity in four of nine dogs. When yohimbine was not administered until 2-3 h after the benzilate,

it antagonized the intoxication in only two of seven dogs. Piperoxan, also an adrenergic blocking agent, was mentioned as being capable of antagonizing intoxication with 3-quinuclidinyl benzilate, but no details of study of its action were given.

Fusek et al. (205) reported that Tacrine inhibited both acetylcholinesterase and butyrylcholinesterase in dog lungs and heart and slightly inhibited acetylcholinesterase in the brain, especially in basal ganglia. Most of the change in the acetylcholinesterase activity of the brain was found to be in the microsomal fraction. Butyrylcholinesterase in the bodies of ganglion cells was said to be completely inhibited in all regions of the brain. These authors found that an intramuscular dose of tacrine at 10 mg/kg completely abolished within 2 h the signs of intoxication induced by an intramuscular injection of 3-quinuclidinyl benzilate at 50 μ g/kg.

Sram et al. (206) reported the results of cytogenetic analyses of spermatozoa from mice that had been given intraperitoneal 3-quinuclidinyl benzilate in single injections at 40 mg/kg or 15 daily doses at 10 mg/kg, of bone marrow cells from mice that had received 15 daily doses at 2 or 10 mg/kg, and of human lymphocytes that had been exposed in vitro at 10^{-4} - 10^{-3} M. They looked also for reverse mutations in yeast cells exposed in vitro at 5×10^{-4} M and 5×10^{-2} M, for host-mediated transformations in yeast cells injected into the peritoneal cavities of mice that were then given 50 or 100 mg/kg subcutaneously, and for dominant-lethal effects in mice after single intraperitoneal injections at 10 or 40 mg/kg or repeated injections--15 at 2 mg/kg, 15 at 10 mg/kg, or 5 at 20 mg/kg. The yeast cells exposed to 3-quinuclidinyl benzilate in vitro gave evidence of weak mutagenic activity. The only other positive results were in bone marrow cells of mice given doses of 10 mg/kg or more. Sram et al. (206) concluded that, because the frequency of chromosomal breaks was not dose-related above the threshold dose for the occurrence of breaks, the breaks were probably due to systemic toxic activity by the benzilate, rather than to any specific mutagenic effect, and that the compound has no serious genetic risk for man.

In another paper, Sram (207) examined the production of chromosomal abnormalities in bone marrow from male Chinese hamsters given single intraperitoneal injections of 3-quinuclidinyl benzilate at 0.1, 1, 10, 20, 40, or 60 mg/kg, dissolved in dimethyl sulfoxide. Control hamsters received dimethyl sulfoxide alone. Doses of 1 mg/kg or more induced more chromosomal gaps and breaks per cell than the solvent. Doses of 1-40 mg/kg yielded apparently dose-related increases in the incidence of chromosomal abnormalities: 0.012 per cell with 1 mg/kg, 0.064 per cell with 10 mg/kg, 0.224 per cell with 20 mg/kg, and 0.292 per cell with 40 mg/kg. The highest dose, 60 mg/kg, produced only 0.076 per cell. Sram considered, on the basis of the count with the highest dose, that the incidence of chromosomal gaps and breaks was not dose-related, but that is questionable for the doses between 1 and 40 mg/kg. However, as Sram pointed out, chromosomal gaps and breaks seem to be evidence of toxic damage to the individual, rather than of heritable change, so they probably do not indicate a serious genetic risk for man.

Hughes et al. (208) gave 10 beagles intravenous 3-quinuclidinyl benzilate at 3 mg/kg twice a week for 3 wk. Five control dogs were given 0.1 N HCl, corresponding in volume and pH to the solution of benzilate at 0.1 ml/kg. Some animals from each group were killed 72 h after the last dose of benzilate, and the others were killed 60 d after the last dose. No lesions attributable to benzilate were found in the testes of the dogs that had been given that compound.

DIETHYLAMINOETHYL BENZILATE

The only other member of the group of esters of benzoic acid on whose effects there is a considerable body of information is diethylaminoethyl benzilate. One review of its pharmacology has been mentioned previously (181); it concerned the general pharmacology of the drug as a peripherally effective muscarinic blocking agent. In addition to having cholinergic spasmolytic activity, diethylaminoethyl benzilate also has mydriatic effectiveness, is able to antagonize barium-induced contraction of intestinal smooth muscle, has local anesthetic properties, and has an antiarrhythmic action on the heart similar to that of quinidine. In 1955, a series of papers established that diethylaminoethyl benzilate affects the functioning of the nervous system in the cat (209), the rat (210), and man (211).

Berger et al. (212) studied the toxicity of single doses of diethylaminoethyl benzilate in mice and monkeys and its general actions on several functional systems. In the monkey, an intravenous dose of 1 mg/kg resulted in mydriasis and some decrease in voluntary movement. A dose of 2 mg/kg induced ataxia with occasional convulsive jerks. A dose of 6 mg/kg induced intermittent clonic convulsions in one monkey; the convulsive state lasted for about 10 min and was followed by a depressed state lasting for over an hour. Pupillary dilatation, the effect of longest duration, sometimes persisted for as long as 20 h.

Diethylaminoethyl benzilate injected intraperitoneally into mice with hexobarbital at 100 mg/kg (212) prolonged anesthesia due to the hexobarbital: benzilate at 10 mg/kg increased the duration of anesthesia by 35.9 min (124%) and at 20 mg/kg increased it by 68.3 min (236%). When diethylaminoethyl benzilate was administered to mice by mouth at 24 mg/kg and the mice were then subjected to what was usually a nonlethal convulsigenic electric shock, 50% of the mice died. The only antimuscarinic effect detectable in these mice was mydriasis. This dose of diethylaminoethyl benzilate did not prolong the latency to onset of convulsion after delivery of the electric shock; a larger dose, 37 mg/kg, did prolong the latency significantly.

When different agonists (acetylcholine, 5-hydroxytryptamine, and histamine) were used (212) to stimulate the smooth muscle in isolated rat colon, diethylaminoethyl benzilate had some ability to antagonize the stimulant action of all three agonists. Its ability to block the action of acetylcholine was about 20 and 100 times its activity against the stimulant actions of 5-hydroxytryptamine

and histamine, respectively. Diethylaminoethyl benzilate did not increase the urinary excretion by the rat of 5-hydroxyindoleacetic acid, but did magnify the pressor action of epinephrine and reduced its LD₅₀. The compound appears, therefore, not to affect the metabolism of serotonin, but to alter in some way the relation between epinephrine and its receptors.

Diethylaminoethyl benzilate had no significant effect on the knee-jerk or flexor reflex in the cat (212); when injected into curarized cats, it altered the predominant frequency of the EEG from 40 per second to 8-15 per second and induced enlarged discharges. The changes in the potentials recorded from the brain were seen in records from both cortical and subcortical regions. Electric arousal consequent to peripheral or thalamic stimulation of cerebral afferent fibers was blocked by diethylaminoethyl benzilate, but recruitment of cortical neurons by stimulation of the thalamus was not affected.

Giarman and Pepeu (75) reported in 1962 that diethylaminoethyl benzilate differed from atropine in that a dose equimolar with atropine at 50 mg/kg (which reduced the concentration of acetylcholine in rat brain by 33%) caused no significant change in the brain's concentration of acetylcholine. Both atropine and diethylaminoethyl benzilate were said to induce mild excitation, however. In contradiction of this report, Frances and Jacob (81) published a graph indicating that intraperitoneal atropine at 1 to 7 or 8 mg/kg had a greater effect in lowering the brain acetylcholine concentration in the mouse than doses of diethylaminoethyl benzilate in the same range, but that the benzilate at 25-30 mg/kg induced a greater lowering of the acetylcholine concentration than atropine.

McColl and Rice (213) found that intraperitoneal injection of mice with diethylaminoethyl benzilate at 10 mg/kg 15 min before similar administration of tremorine increased the ED₅₀ of tremorine from 5.8 to 38.0 mg/kg. Such an effect can be explained as being due to depletion by the benzilate of the store of acetylcholine in the brain cells.

In 1964, Jacobsen published a review of the literature through 1962 on the actions of diethylaminoethyl benzilate (214). It reported that oral doses of this benzilate of up to 80 mg had been tolerated by man, but that daily doses of about 90 mg had given rise to mild arrhythmias and cyanosis. The compound had been found to enter the rat brain in unaltered form. When ¹⁴C-labeled diethylaminoethyl benzilate had been administered to rats, 85% of the label had been recovered in the urine within 24 h. Nothing was known at that time about the nature of the metabolites. The compound had been found to inhibit oxidative phosphorylations in brain cells in vitro, and, at high concentrations (10⁻³ M), to inhibit monoamine oxidase. In man, doses of 40-200 mg of this benzilate had been found to reduce excretion of 5-hydroxyindoleacetic acid to zero. Thus, the compound interferes in some way with the utilization or metabolism of serotonin, and presumably with the function of serotonergic synapses. In addition to having peripheral antimuscarinic activity, graded by various

investigators as between 0.01 and 0.33 that of atropine, diethylaminoethyl benzilate had been found to be particularly potent in blocking transmission through the ascending reticular system; it had also been found to affect the descending reticular system, but not to be useful in treating parkinsonism, because of its other central effects. Diethylaminoethyl benzilate inhibits the metabolism of barbiturates and potentiates their central depressant activity. In addition to inducing hyperactivity in mice and rats and reducing the reactions to stress in rats and cats, diethylaminoethyl benzilate had been found not to affect sham rage, to inhibit fighting between rats exposed to electric shocks, and to decrease the rate of learning of complicated tasks (tasks requiring discrimination between stimuli and the making of different responses to different stimuli) in mice, rats, and cats.

Berger et al. (80) reported that diethylaminoethyl benzilate was particularly effective in increasing the duration of after-discharges by the cat hippocampus after a weak, brief electric shock, being 40-100 times as active as atropine in this regard. As to its antispasmodic action in vitro and its ability to block salivation induced by intracranial injection of 80 ug of acetylcholine chloride into a mouse, diethylaminoethyl benzilate was 4% and 77%, respectively, as potent as atropine.

Shitov (215) found that benactyzine and two other anticholinergic compounds increased the action of magnesium sulfate on the EEG of the rabbit in proportion to the activity of the compounds in activating cholinesterase.

Banshchikov and Stoliarov (216) reviewed a group of substances that had been found to be capable of evoking mental disturbances. All the substances have marked anticholinergic activity, and diethylaminoethyl benzilate was among them. The others are Biel's compounds, JB-318, JB-329 (Ditran), and JB-336.

The authors (216) stated that usual medicinal doses of diethylaminoethyl benzilate not infrequently produce mental changes--retardation or stoppage of the current of thought, a feeling of emptiness, forgetfulness, shortened attention span, sensations of heaviness and altered shape of the limbs, apathy, sluggishness, impression of isolation from the environment, hostility, apprehension, horror, anxiety, and hypochondriasis. A relatively brief period of these effects might be followed by a period of euphoria if more than 5 mg of the benzilate had been taken.

The first report of a psychotomimetic episode due to diethylaminoethyl benzilate in a human being was credited to Vojtechovsky (217) by Banshchikov and Stoliarov (216). This investigator had published in 1958 a case report on a female physician who had accidentally taken about 1.4 g of the benzilate. The woman experienced visual hallucinations and brief delirium. Intentional administrations of diethylaminoethyl benzilate to human subjects had shown (218) that the psychotomimetic state lasted for 4-12 h and was accompanied by a decrease in urinary excretion of 5-hydroxyindoleacetic acid. These authors stated that

diethylaminoethyl benzilate had been found to inhibit monoamine oxidase only slightly less strongly than iproniazid. As the psychotomimetic state waned, the urinary excretion of 5-hydroxyindoleacetic acid increased to such an extent that the 24-h excretion of this degradation product of serotonin was within normal limits. It had been found in the same experiment that the urinary excretion of 17-ketosteroids was markedly reduced during the period of psychosis and that the activity rebounded at about the same time as the urinary excretion of 5-hydroxyindoleacetic acid. In the case of the 17-ketosteroids, however, the rebound increased the total daily urinary excretion of 17-ketosteroids by nearly 50%.

Edelson *et al.* (219) used [^3H]diethylaminoethyl benzilate to study the absorption, distribution, and metabolic fate of this benzilate in the rat. The substance used was randomly labeled. After intraperitoneal injection of benzilate at about 82.5 mg/kg, ^3H was detectable in the blood at a fairly high concentration about 12 min after injection; the blood concentration was highest about 36 min after injection. At 5 h after injection, the ^3H concentration was about 28% of its peak. The authors estimated the half-time of the label in the blood to be about 80 min. If one calculates (from data in the paper) the time required for removal of half the peak ^3H concentration from the blood, that time is found to be about 114 min after the injection. The authors' value of 80 min for the half-time would require the concentration of label in the blood 5 h after injection to be about 6.7% of the peak concentration, whereas it was actually about 28% of the peak. The estimate of 114 min is believed to be a better value for the half-time.

Within 24 h after injection, 44.8% of the label was recovered in the urine and 41.6% in the feces and the gastrointestinal tract and its contents. The carcass contained 14.2% of the label, of which the greatest amount was in the liver. The expired air contained about 0.3% of the ^3H , in the form of water. The urine contained the unchanged compound, ethylaminoethyl benzilate, and benzoic acid. Apparently, a considerable portion of the diethylaminoethyl benzilate had been hydrolyzed to release benzoic acid, which accounted for 54.4% of the label in the urine. A smaller portion of the original compound had been monodealkylated to ethylaminoethyl benzilate, which accounted for 13.8% of the label in the urine. This dealkylation may have contributed the small amount of the label found in the water of the expired air. The fate of the diethylaminoethanol that must have been released by hydrolysis of the original compound remains unknown. A study of the hydrolysis *in vitro* of the original benzilate at a pH of 7.4 during incubation at 37°C revealed that approximately 50% of the compound underwent spontaneous hydrolysis within 5 h.

Sram (207) gave Chinese hamsters diethylaminoethyl benzilate intraperitoneally at 0.1, 1, and 10 mg/kg. The animals were killed 24 h later, and the cells of their bone marrow were examined for chromosomal abnormalities. The lowest dose of the benzilate did not give rise to any detectable chromosomal abnormalities; 1 mg/kg resulted in a 50% increase